(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 July 2003 (03.07.2003)

PCT

(10) International Publication Number
WO 03/053158 A2

(51) International Patent Classification[7]: A23J 3/08, 3/22, A23L 1/30, 1/24, A23C 21/02, 15/16, 9/137, A61K 47/42

(21) International Application Number: PCT/CA02/01988

(22) International Filing Date:
20 December 2002 (20.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/341,232 20 December 2001 (20.12.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/053158 A2

(54) Title: MALLEABLE PROTEIN MATRICE AND USES THEREOF

(57) Abstract: The present invention relates to a malleable protein matrice (MPM), which is the reaction product of the agglomeration of proteins after a fermentation process and is exhibiting biological activities and is suitable for the incorporation (or encapsulation) of various hydrophilic or lipophylic substances. The present invention also relates to the process for the preparation of the malleable protein matrice and its usages.

constitutes a pollutant *per se*, the small manufacturers have therefore to spend money to discard the serum lactis which is mainly used for animal feed.

Simpler and less costly processes were developed to retrieved whey proteins but with also with concomitant drawbacks. Methods using temperature, pH, salt, enzymes, fermentation and flocculent are among the main parameters used to help the retrieval of whey proteins but generally lead to isolates exhibiting poor commercial quality and value. Patent CA 2,247,824 by Lewandoski and co-inventors describes a process for the production of microbial biomass from the effluent of dairy products. The resulting biomass from that process is used for animal feeding only. However, this product is not having functional properties such as emulsifiant properties that are needed for applications in human food.

Many processes and methods are offered to replace fat in food products. Agglomerates of whey proteins are used to replace fat like as described in U.S. Patent 5,358,730. The process involves a thermal treatment of whey proteins at a pH above their isoelectric point with the addition of salt. The process leads to the formation of curds (solid gels that can be shopped off in little pieces) that can be used in fat replacement. Whey proteins are extensively used in the food industry for their functional properties. However, this product is a solide and non-malleable product that is difficult to use in most of the food, cosmetic, pharmaceutical and nutraceuticals applications.

Proteins are also excellent film formers, conditioning agents, and moisturizers for hair and skin. However, natural proteins generally have limited use in cosmetics and toiletries because they are somewhat unstable and tend to precipitate or denature when exposed to high temperatures or salt solutions. In addition they are often hydrolyzed by chemical reagents or acids and bases. Even if these difficulties are overcome, the formulation of cosmetic products containing proteins is further fraught with difficulty since each protein has an isoelectric point i.e. a pH at which the protein is neutral. If it is desired to form compositions having a pH which is below the isoelectric point of the protein, the protein may possibly form an insoluble precipitate.

Furthermore, a large number of food products like mayonnaise, dressings, margarine, spreads or low-fat or zero-fat substitutes, can be stabilized by polysaccharides as emulsion stabilizers or thickening agents. Also in the medical, pharmaceutical and cosmetic fields, polysaccharides they are used as emulsion stabilizers. Well known polysaccharides are obtained from a variety of plant seeds, e.g. guar gum from Cyamopsis tetragonaloba (guar) or locust bean gum (LBG) from locust bean. Other well-known sources are seaweed, giving carrageenan, alginates or agar.

The use of polysaccharides and proteins in cosmetic compositions is well known in the art. Polysaccharides are known to be good humectants, film formers, and function as skin moisturizers. Certain polysaccharides also have gelling ability and are useful in formation of higher viscosity liquid or solid, compositions. However, polysaccharides may tend to provide a heavy, sticky feel on the skin and, when used in quantities sufficient to cause gelling, may provide products which are not aesthetically pleasing.

Food science

The process described in the U.S. Patent 4,699,793 is used to produce seasoning. Because of the heat treatment performed before the fermentation, the resulting product has an undesirable taste and a poor homogeneity, which are the most important parameters in food science.

It is known that the presence of certain bacteria is associated with numerous beneficial effects on health (Gomes *et al.* (1999) *T. Food Sc. & Tech.* **10**:139-157). The microorganisms are present in many foods and are frequently used as probiotics to improve some biological functions in the host. Clinical trials have demonstrated that selected probiotic strains can influence the composition of the intestinal microflora and modulate the host immune system. Pre-, pro- and synbiotics offer both protection against and cure a variety of endemic and acute diseases.

More particularly, the lactic acid bacteria (LAB) are known for their several beneficial effects on health. Perdigon et al. (Curr Issues Intest Microbiol., 2001, Mar 2(1):27-42), have proceed with an important review of the lactic bacteria on health, particularly on immune system. The activation of the systemic and secretory immune response by LAB requires

many complex interactions among the different constituents of the intestinal ecosystem (microflora, epithelial cells and immune cells). Through different mechanisms they send signals to activate immune cells. Thus the knowledge of the normal intestinal microflora, the contribution of LAB and their role in the numerous functions in the digestive tract as well as the functioning of the mucosal immune system form the basis for the study and selection of a probiotic strain with immunostimulatory properties. In the selection of LAB for their immunostimulatory capacity it helps to know not only the effect which they have on the mucosal immune system, but the specific use to which these oral vaccine vectors are being put.

Pharmaceutical

Delivery of therapeutic agents to a mammalian host can frequently be as important as the activity of the drug in providing effective treatment. For the most part, drugs are delivered orally, frequently initially at a dosage below the therapeutic dosage and by repetitive administration of the drug, the dosage is raised to a therapeutic level or a level exceeding the therapeutic level. In many cases, the fact of having a dosage above therapeutic level provides for adverse effects, since most drugs are not only effective for the intended purpose, but frequently have adverse side effects. Various proposals have been made to avoid these problems, such as slow-release capsules, depots, pumps, and the like. These various approaches have numerous short comings for general applications where one wishes to maintain the presence of a therapeutic agent at a therapeutic dosage for an extended period. Invasive procedures are frequently undesirable, requiring surgery for introduction of the delivery device, followed by subsequent removal. Where the delivery device is placed on the skin, the agent must be capable of transport across the skin at the desired rate. Slow release particles have a limited time span and when introduced into the blood stream will be rapidly phagocytosed.

Oral administration in the form of a conventional tablet, pill or capsule constitutes the generally preferred route for administration of pharmaceuticals since this route is generally convenient and acceptable to patients. Unfortunately such compositions may be associated with certain disadvantages, particularly in the treatment of pediatric or geriatric

patients, who may dislike or have difficulty in swallowing such compositions, or where administration of a conventional tablet, pill or capsule is not duable.

The field of biodegradable polymers has developed rapidly since the synthesis and biodegradability of polylactic acid was first reported by Kulkami et al., 1966 "Polylactic acid for surgical implants" Arch. Surg., 93:839, Several other polymers are known to biodegrade, including polyanhydriques and polyorthoesters, which take advantage of labile backbone linkages, as reported by Heller et al., 1990, Biodegradable Polymers as Drug Delivery Systems; Chasin, M. and Langer, R., Eds., Dekker, New York, 121-161. Since it is desirable to have polymers that degrade into naturally occurring materials, polyaminoacids have been synthesized for *in vivo* use. This was the basis for using polyesters of alpha-hydroxy acids (viz., lactic acid, glycolic acid), which remain the most widely used biodegradable materials for applications ranging from closure devices (sutures and staples) to drug delivery systems (U.S. Pat. No. 4,741,337 to Smith et al.; Spilizewski et al., 1985 "The effect of hydrocortisone loaded poly(dl-lactide) films on the inflammatory response, " J. Control. Rcl. 2:197-203). Despite the development of novel biodegradable polymers, there is still a need for inexpensive and efficient delivery systems.

Exopolysaccharides act as biological response modifier as reported by Ruiz-Bravo A. (Clinical and Diagnostic Laboratory Immunology 2001, Jul; 8(4)-706-10). U.S. patents 5,888,552; 5,456,924; 5,451,412; 5,290,571; 5,230,902 describe compositions and methods to improve immune responses at large either for cancer or HIV-patients. U.S. patent 5,888,552 describes anti-cancer therapeutic compositions containing whey proteins while U.S. 5,456,924 describes a method of treatment of HIV-seropositive individual with dietary whey proteins.

It would be highly desirable to be provided with a biodegradable and non-toxic malleable protein matrice and a process to produce such that would turn or convert an industrial waste into a product with a commercial value and a biological activity.

SUMMARY OF THE INVENTION

One object of the present invention is to provide a biodegradable and non-toxic malleable protein matrice (MPM).

Preferably, the invention relates to matrice of whey proteins and exopolysaccharides. In addition, the matrice of the present invention is advantageously used to replace fat or for the incorporation or encapsulation of various hydrophilic or lipophylic substances and particularly substances used in the food, cosmetic, nutraceuticals and pharmaceutical sectors.

Another object of the present invention, there is to provide a novel method for the retrieval of whey proteins from the serum lactis which leads to a new kind of whey protein-based product. This new product is referred hereto as a malleable protein matrice (MPM), which is the reaction product of the agglomeration of whey proteins present in the serum lactis after a fermentation process. It has the texture of a malleable cream exhibiting biological activities and unique properties for the incorporation (or encapsulation) of various hydrophilic or lipophylic substances.

It is also an object of the invention to prepare various types of MPMs with different properties, characteristics and multiple applications and to prepare them directly from an industrial waste (whey or serum lactis).

In accordance with the present invention, there is provided a malleable protein matrix comprising:

- a precipitate of a protein of interest in solution;

- at least one microorganism capable of fermenting the solution containing the protein; and

-a matrix carrier allowing fermentation of the protein and the microorganism.

The matrix in accordance with a preferred embodiment of the present invention, wherein the fermentation is promoted by co-culture of at least two microorganisms simultaneously or successively.

The matrix in accordance with a preferred embodiment of the present invention, further comprising a fermentation by-products of the fermentation of the solution containing the protein by the microorganism.

The matrix in accordance with a preferred embodiment of the present invention, further comprising a peptide.

The matrix in accordance with a preferred embodiment of the present invention, wherein the peptide comprises at least two amino acid residues.

The matrix in accordance with a preferred embodiment of the present invention, wherein the peptide comprises more than one hundred amino acid residues.

The matrix in accordance with a preferred embodiment of the present invention, further comprising components obtained during agglomeration of the protein.

The matrix in accordance with a preferred embodiment of the present invention, further comprising components present in aqueous phase.

The matrix in accordance with a preferred embodiment of the present invention, wherein the protein is selected from the group consisting of natural protein, plant protein, animal derived protein and synthetic protein.

The matrix in accordance with a preferred embodiment of the present invention, wherein the protein is selected from the group consisting of albumen, amylase, amyloglucosidase, arginine/lysine polypeptide, casein, catalase, collagen, crystalline, cytochrome C, deoxyribonuclease, elastin, fibronectin, gelatin, gliadin, glucose oxidase, glycoproteins, hexyldecyl ester of hydrolyzed collagen, human placental protein, human placental enzymes, iodized corn protein, keratin, lactalbumine, lactoferrin, lactoglobulin, lactoperoxidase, lipase, milk protein, hyristoyl glycin/histidine/lysin polypeptide, nisin, oxido reductase, pancreatin, papaïne, pepsin, placental protein, protease, saccharomyces polypeptides, serum albumin, serum protein, silk, sodium stearoyl lactalbumin, soluble proteoglycan, soybean palmitate, soy, egg, peanut, cottonseed, sunflower,

pea, whey, fish, seafood, subtilisin, superoxide dismutase, sutilains, sweet almond protein, urease, wheat germ protein, wheat protein, whey protein, zein and hydrolyzed vegetable protein.

The matrix in accordance with a preferred embodiment of the present invention, wherein the protein is whey protein.

The matrix in accordance with a preferred embodiment of the present invention, wherein the fermentation by-products is polysaccharide.

The matrix in accordance with a preferred embodiment of the present invention, wherein the polysaccharide is selected from the group of exopolysaccharide and anionic polysaccharide.

The matrix in accordance with a preferred embodiment of the present invention, wherein the polysaccharide contains at least four saccharide moieties.

The matrix in accordance with a preferred embodiment of the present invention, wherein the saccharide moieties are selected from the group consisting of D and L forms of glucose, fructose, xylose, arabinose, fucose, galactose, pyruvic acid, succinic acid, acetic acid, 3,6-anhydrogalactose sulfate, galactose-4-sulfate, galactose-2-sulfate, galactose-2, 6-disulfate, mannose, glucuronic acid, mannuronic acid, guluronic acid, galactouronic acid, and rhamnose.

The matrix in accordance with a preferred embodiment of the present invention, wherein the polysaccharide have molecular weight ranging from about 500 to about 15,000,000 daltons.

The matrix in accordance with a preferred embodiment of the present invention, wherein the molecular weight is ranging from about 5,000 to 6,000,000 daltons.

The matrix in accordance with a preferred embodiment of the present invention, wherein the molecular weight is ranging from about 25,000 to 1,000,000 daltons.

The matrix in accordance with a preferred embodiment of the present invention, wherein the polysaccharide is selected from the group consisting of heteropolysaccharides, homopolysaccharides, galactans,

galactomannans, glucomannans, polyuronic acids, dextran sulfate, heparin, pectin, sodium alginate and mixtures thereof.

The matrix in accordance with a preferred embodiment of the present invention, wherein galactan is selected from the group consisting of agar, agarose, kappa-carageenan, iota carageenan and lambda carageenan.

The matrix in accordance with a preferred embodiment of the present invention, wherein galactomannan is selected from the group consisting of locust bean gum and guar.

The matrix in accordance with a preferred embodiment of the present invention, wherein glucan is selected from the group consisting of cellulose and derivatives thereof, starch and derivatives, dextrans, pullulan, beta 1,3-glucans, chitin, xanthan and tamatind.

The matrix in accordance with a preferred embodiment of the present invention, wherein glycomannan is konjac.

The matrix in accordance with a preferred embodiment of the present invention, wherein polyuronic acid is selected from the group consisting of algin, alginate and pectin.

The matrix in accordance with a preferred embodiment of the present invention wherein heteropolysaccharide is selected from the group consisting of gellan, welan, gum arabic, karaya gum, okra gum, aloe gum, gum tragacanth, gum ghatti quicessed gum , psyllium and starch arabinogalactan.

The matrix in accordance with a preferred embodiment of the present invention, wherein the microorganism is selected from the group consisting of *Bifidobacterium adolescentis*, *Bifidobacterium angulatum*, *Bifidobacterium animalis*, *Bifidobacterium asteroides*, *Bifidobacterium bifidum*, *Bifidobacterium boum*, *Bifidobacterium breve*, *Bifidobacterium catenulatum*, *Bifidobacterium choerinum*, *Bifidobacterium coryneforme*, *Bifidobacterium cuniculi*, *Bifidobacterium dentium*, *Bifidobacterium gallicum*, *Bifidobacterium gallinarum*, *Bifidobacterium indicum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium longum* DJO10A, *Bifidobacterium longum* NCC2705, *Bifidobacterium magnum*,

Bifidobacterium merycicum, *Bifidobacterium minimum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium pseudolongum*, *Bifidobacterium pseudolongum subsp. globosum*, *Bifidobacterium pullorum*, *Bifidobacterium ruminantium*, *Bifidobacterium saeculare*, *Bifidobacterium scardovii*, *Bifidobacterium subtile*, *Bifidobacterium suis*, *Bifidobacterium thermacidophilum*, *Bifidobacterium thermacidophilum subsp. suis*, *Bifidobacterium thermophilum*, *Bifidobacterium urinalis*, *Lactobacillus acetotolerans*, *Lactobacillus acidipiscis*, *Lactobacillus acidophilus*, *Lactobacillus agilis*, *Lactobacillus algidus*, *Lactobacillus alimentarius*, *Lactobacillus amylolyticus*, *Lactobacillus amylophilus*, *Lactobacillus amylovorus*, *Lactobacillus animalis*, *Lactobacillus arizonensis*, *Lactobacillus aviarius*, *Lactobacillus bifermentans*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus casei*, *Lactobacillus cellobiosus*, *Lactobacillus coleohominis*, *Lactobacillus collinoides*, *Lactobacillus coryniformis*, *Lactobacillus coryniformis subsp. coryniformis*, *Lactobacillus coryniformis subsp. torquens*, *Lactobacillus crispatus*, *Lactobacillus curvatus*, *Lactobacillus cypricasei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus delbrueckii subsp. delbrueckii*, *Lactobacillus delbrueckii subsp. lactis*, *Lactobacillus durianis*, *Lactobacillus equi*, *Lactobacillus farciminis*, *Lactobacillus ferintoshensis*, *Lactobacillus fermentum*, *Lactobacillus fornicalis*, *Lactobacillus fructivorans*, *Lactobacillus frumenti*, *Lactobacillus fuchuensis*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, *Lactobacillus graminis*, *Lactobacillus hamsteri*, *Lactobacillus helveticus*, *Lactobacillus helveticus subsp. jugurti*, *Lactobacillus heterohiochii*, *Lactobacillus hilgardii*, *Lactobacillus homohiochii*, *Lactobacillus intestinalis*, *Lactobacillus japonicus*, *Lactobacillus jensenii*, *Lactobacillus johnsonii*, *Lactobacillus kefir*, *Lactobacillus kefiri*, *Lactobacillus kefiranofaciens*, *Lactobacillus kefirgranum*, *Lactobacillus kimchii*, *Lactobacillus kunkeei*, *Lactobacillus leichmannii*, *Lactobacillus letivazi*, *Lactobacillus lindneri*, *Lactobacillus malefermentans*, *Lactobacillus mali*, *Lactobacillus maltaromicus*, *Lactobacillus manihotivorans*, *Lactobacillus mindensis*, *Lactobacillus mucosae*, *Lactobacillus murinus*, *Lactobacillus nagelii*, *Lactobacillus oris*, *Lactobacillus panis*, *Lactobacillus pantheris*, *Lactobacillus parabuchneri*, *Lactobacillus paracasei*, *Lactobacillus paracasei subsp. paracasei*,

Lactobacillus paracasei subsp. tolerans, *Lactobacillus parakefiri*, *Lactobacillus paralimentarius*, *Lactobacillus paraplantarum*, *Lactobacillus pentosus*, *Lactobacillus perolens*, *Lactobacillus plantarum*, *Lactobacillus pontis*, *Lactobacillus psittaci*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus ruminis*, *Lactobacillus sakei*, *Lactobacillus sakei* L45, *Lactobacillus salivarius*, *Lactobacillus salivarius subsp. salicinius*, *Lactobacillus salivarius subsp. salivarius*, *Lactobacillus sanfranciscensis*, *Lactobacillus sharpeae*, *Lactobacillus sp.* NGRI 0001, *Lactobacillus suebicus*, *Lactobacillus thermotolerans*, *Lactobacillus vaccinostercus*, *Lactobacillus vaginalis*, *Lactobacillus vermiforme*, *Lactobacillus versmoldensis*, *Lactobacillus zeae*, *Lactococcus garvieae*, *Lactococcus lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. hordniae*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. lactis bv. diacetylactis*, *Lactococcus piscium*, *Lactococcus plantarum*, *Lactococcus raffinolactis*, *Leuconostoc argentinum*, *Leuconostoc carnosum*, *Leuconostoc citreum*, *Leuconostoc fallax*, *Leuconostoc ficulneum*, *Leuconostoc fructosum*, *Leuconostoc gasicomitatum*, *Leuconostoc gelidum*, *Leuconostoc inhae*, *Leuconostoc kimchii*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Leuconostoc mesenteroides subsp. cremoris*, *Leuconostoc mesenteroides subsp. dextranicum*, *Leuconostoc mesenteroides subsp. mesenteroides*, *Leuconostoc mesenteroides subsp. mesenteroides* ATCC 8293, *Leuconostoc pseudomesenteroides*, *Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium australiense*, *Propionibacterium avidum*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionibacterium freudenreichii subsp. freudenreichii*, *Propionibacterium freudenreichii subsp. shermanii*, *Propionibacterium granulosum*, *Propionibacterium jensenii*, *Propionibacterium lymphophilum*, *Propionibacterium microaerophilum*, *Propionibacterium propionicum*, *Propionibacterium thoenii*, *Saccharomyces delbrueckii*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Saccharomyces globosus*, *Saccharomyces carlsbergensis*, *Kluyveromyces fragilis*, *Kluyveromyces bulgaricus*, *Kluyveromyces lactis*, *Torula holmii*, *Candida tenuis*, R2C2, INIX, ES1 and K2.

The matrix in accordance with a preferred embodiment of the present invention, wherein the microorganism is selected from the group consisting of *L. rhamnosus, L. acidphilus, L. casei, L. lactis, L. plantarum, L. Kefirgranum,* R2C2, INIX, ES1 and K2.

The matrix in accordance with a preferred embodiment of the present invention, wherein the microorganism is R2C2.

The matrix in accordance with a preferred embodiment of the present invention, wherein the microorganism is INIX.

The matrix in accordance with a preferred embodiment of the present invention, wherein the microorganism is *L. Kefirgranum.*

The matrix in accordance with a preferred embodiment of the present invention, wherein the microorganism is *bacillaceae, bifidobacteriaceae, enterobacteriaceae, enterococcaceae, lactobacillaceae; propionibacteriaceae* and yeast.

The matrix in accordance with a preferred embodiment of the present invention, wherein the bacillaceae is *Bacillus subtilis.*

The matrix in accordance with a preferred embodiment of the present invention, wherein the bifidobacteriaceae is one selected from the group consisting of *Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis* and *Bifidobacterium lactis.*

The matrix in accordance with a preferred embodiment of the present invention, wherein the *enterobacteriaceae* is *Escherichia coli* Nissle 1917.

The matrix in accordance with a preferred embodiment of the present invention, wherein the *enterococcaceae* is *Enterococcus faecium.*

The matrix in accordance with a preferred embodiment of the present invention, wherein the *lactobacillaceae* is one selected from the group consisting of *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus crispatus, Lactobacillus fermentum, Lactobacillus johnsonii, Lactobacillus paracasei, Lactobacillus plantarum, Lactobaillus reuteri, Lactobacillus rhamnosus* and *Lactobacillus salivarius.*

The matrix in accordance with a preferred embodiment of the present invention, wherein the yeast is *saccharomyces cerevisiae boulardii*.

In accordance with the present invention, there is provided a microorganism R2C2 isolated from a consortium obtained from Kefir grain.

In accordance with the present invention, there is provided a microorganism K2 isolated from a consortium obtained from Kefir grain.

In accordance with the present invention, there is provided a microorganism ES1 isolated from a consortium obtained from Kefir grain.

In accordance with the present invention, there is provided a microorganism INIX isolated from ATCC 43761 strain.

In accordance with the present invention, there is provided a process for manufacturing the matrix of the present invention, the process comprising the steps of:

a) fermenting a protein solution with a microorganism in a medium;

b) precipitating protein from the proteins solution of step a); and

c) isolating precipitated proteins from supernatant.

The process in accordance with a preferred embodiment of the present invention, wherein the fermenting step is promoted by co-culturing at least two microorganisms simultaneously or successively.

The process in accordance with a preferred embodiment of the present invention, wherein the process further comprises a step between steps a) and b) for addition of a polysaccharide.

The process in accordance with a preferred embodiment of the present invention, wherein the process further comprises a step between steps b) and c) for addition of a polysaccharide.

The process in accordance with a preferred embodiment of the present invention, further comprising a step of pasteurization of the

proteins solution before step a). This process can further include a sterilization step after the pasteurization step.

The process in accordance with a preferred embodiment of the present invention, wherein precipitation of fermented proteins is effected by at least one method selected from the group consisting of salt addition, pH modulation, thermal treatment, proteolytic enzymes addition and floculent addition.

The process in accordance with a preferred embodiment of the present invention, wherein the flocculent is a bacterial flocculent.

The process in accordance with a preferred embodiment of the present invention, wherein the bacterial flocculent is *L. Kefirgranum*.

The process in accordance with a preferred embodiment of the present invention, wherein separation of precipitated proteins from supernatant is effected by a method selected from the group of centrifugation and filtration.

In accordance with the present invention, there is provided a composition comprising the matrix of the present invention in association with a pharmaceutically acceptable carrier.

In accordance with the present invention, there is provided the use of the matrix of the present invention, wherein the use is for the manufacture of a product selected from the group of food product, medical product, pharmaceutical product, cosmetic product, probiotic, functional food and nutraceutical.

In accordance with the present invention, there is provided the use of the matrix of the present invention, wherein the use is for the manufacture of a food product.

The use in accordance with a preferred embodiment of the present invention, wherein the matrix is used as an emulsion stabilizer or thickening agent.

The use in accordance with a preferred embodiment of the present invention, wherein the food product is selected from the group

consisting of mayonnaise, dressing, margarine, spread, butter, whipped cream, cream, yogurt, cheese and low-fat substitute.

The use in accordance with a preferred embodiment of the present invention, wherein the matrix is used as a delivery vehicle.

In accordance with the present invention, there is provided the use of the matrix of the present invention for the preparation of a probiotic.

In accordance with the present invention, there is provided the use of the matrix of the present invention, wherein the use is for cosmetic product.

The use in accordance with a preferred embodiment of the present invention, wherein the cosmetic product is selected from the group consisting of skin lotion, cream, sunscreen, blush, mascara, eyeshadow, shampoo and conditionner.

In accordance with the present invention, there is provided the use of the matrix of the present invention for increasing immune response in a subject.

In accordance with the present invention, there is provided a method of increasing immune response in a subject, comprising administering an effective amount of the matrix of the present invention to the subject.

In accordance with the present invention, there is provided the use of the matrix of the present invention for reducing triglyceride level in a subject.

In accordance with the present invention, there is provided a method for reducing triglyceride level in a subject, comprising administering an effective amount of the matrix of the present invention to the subject.

In accordance with the present invention, there is provided the use of the matrix of the present invention for reducing TNF-α level in a subject.

In accordance with the present invention, there is provided a method for reducing TNF-α level in a subject, comprising administering an effective amount of the matrix of the present invention to the subject.

In accordance with the present invention, there is provided the use of the matrix of the present invention for increasing gluthatione level in a subject.

In accordance with the present invention, there is provided a method for increasing gluthatione level in a subject, comprising administering an effective amount of the matrix of the present invention to the subject.

The MPM of the present invention also fulfill a long-felt need in different sectors, namely in food (fat replacement, thickening agent), cosmetic (delivery systems, physiological effects), nutraceuticals, functional food, probiotic and pharmaceutical (oral delivery systems, biological response modifier drug delivery systems).

All the references herein are incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a general schema of the preparation process for MPM;

Fig. 2 illustrates a detailed schema of the matrix formation;

Fig. 3 illustrates the formulation of matrix formulation;

Fig. 4 illustrates one example of an industrial implementation of the present invention; and

Figs. 5A-B illustrate homology between gene ARN165 of strains INIX (SEQ ID NO:1), K2 (SEQ ID NO:2), R2C2 (SEQ ID NO:3), ES1 (SEQ ID NO: 4), ATCC 43761 (SEQ ID NO:5) and ATCC 51647 (SEQ ID NO:6).

DETAILED DESCRIPTION OF THE INVENTION

The invention consists in a malleable protein matrice (MPM) produced from fermented residual whey obtained from the cheese industry. The MPM is obtained by triggering agglomeration of whey proteins, which are then retrieved by various means. The process allows

the production of insoluble and malleable protein matrices composed of 1) proteins and/or peptides, 2) one or several bacterial strains, 3) fermented by-products, 4) other components obtained during the agglomeration and retrieval process of the agglomerates and 5) components present in the aqueous phase. Following the agglomeration, the resulting matrix is retrieved by filtration, centrifugation or with any other methods allowing such retrieval. The protein agglomeration can be triggered by, but not limited to, a modulation of pH, temperature, the addition of salts, the addition of proteolytic enzymes, the addition of flocculent or the combination of all or some of those methods. The invention also describes various parameters that can affect the resulting characteristics of the matrix like the bacterial component of the MPM.

This matrix and its production process present major advantages over the matrice and production processes known in the art. The production process as described below allows the obtention of a uniform formulation directly from lactoserum or other primary protein source when all the components are present prior agglomeration. The components are found either in the agglomerate and the aqueous phase of the post-agglomeration fermentation product. A formulation containing MPM is produced in mixing the MPM and other products to have introduced in the formulation in water, oil or other liquid suitable for such formulation. Another formulation is produced in lyophilizing MPM and hydrating them with a solution containing other products to be introduced in the formulation.

The polymers used can be from different origins, such as from a microorganism, from plant and they also can be synthetic. The polymer is being mixed to the proteins before, during or after the process of agglomeration. The amount of polymers trapped in the matrix may vary to form the resulting matrix. The source of proteins used in the agglomeration process can be from either pure whey obtained from a cheese factory or from a concentrate of whey proteins (WPC, CPI) resuspended in an aqueous solution. The agglomeration process is preceded by a fermentation process or of any other methods to improve the quality of the final product obtained: flavor, color, texture, conservation

time, functional properties, nutritional properties, biological properties, pharmaceutical properties.

Fig. 1 illustrates the preferred embodiment of the process of the present invention consisting in a fermentation process of whey with a pure strain of lactobacillus isolated from a consortium obtained from Kefir grain (R2C2 strain accession number: 041202-3 National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E 3R2). The first step is a pre-culture where freeze-dried, or frozen ferment culture is used to inoculate whey or seed medium suitable for the species used like pure strain of lactobacillus isolated from a consortium obtained from Kefir grain (R2C2 strain). The fermentation is continued to get a concentration of bacteria of 10^8 to 10^9 bacteria per ml of pre-culture. The pre-culture is then inoculated in whey or protein solution in an amount of from 1 to 12.5%. Whey may be used as is, or supplemented with different culture additives suitable for the species used. During the proliferation process, the lactobacillus produces an exopolysaccharide (EPS), which is secreted in the medium, along with some endogenous proteases. The endogenous proteases present in the medium hydrolyze the whey proteins to generate peptides with various length and hydrophobicity. The whey medium is maintained under appropriate culture conditions to promote a rapid multiplication of the microorganisms used. If needed, a constant temperature, pH, agitation, aeration and other culture conditions are supplied. For triggering agglomeration of whey proteins, several means can be used to facilitate or inducing the formation of agglomerates like pH modification, salt addition and heat treatment. Agitation is needed to provide good homogeneity of the resulting matrice which contain microorganisms, peptides, proteins, fermented by-products. Continuous centrifugation is preferred to promote a better homogeneity of the matrice but various retrieval means can also be used.

MPM can be used under a humid form or dried and can be lyophilized or dried by other means and once dried the MPMs are also compressible with a Carver press to form solid tablets. Lyophilized MPMs are compressible without the need to add any excipients to form tablets that could have multiple applications like incorporation of probiotics or

drugs. The tablets hydrate slowly because of their high content of proteins and are protecting the incorporated agents while passing in the stomach environment. MPM can integrate water, oil or other solvent to improve its general properties. The compositions and/or formulations obtained are useful in food science, cosmetic, nutraceuticals, functional food, probiotic and pharmaceuticals.

Fig. 2 illustrates the matrix formation and Fig. 3 illustrates formulations produced with MPM.

The process described to produce MPMs is preferably made of non-concentrated sources of whey proteins like serum lactis. The MPMs exhibit an improved homogeneity and a product with improved functional and organoleptic properties as well as beneficial effects on health because the fermentation process of the present invention is performed in a non-concentrated solution. There is therefore no need to homogenize the resulting matrix with high shear conditions as for the processes known in the art.

Fig. 4 illustrates an example of an industrial implementation of the process of the present invention. As shown in Fig. 4, a preculture medium is prepared with whey and yeast extract, followed by the pasteurization of this preparation. The pasteurized solution is then inoculated with ferment and fermented under control to the obtention of a bacterial culture of 10^8-10^9 bacteria per ml of preculture. One person skilled in the art would understand that the preculture medium preparation does not need to be part of the production process.

Whey is then provided in fermentor to which is added the preculture medium for fermentation. After completion of the fermentation process, the precipitation of the fermented proteins is achieved by one or more of the methods previously described and the precipitated proteins are isolated from the supernatant and stored until delivery.

The MPMs described above have multiple applications that are listed below. MPMs is an inexpensive product with a variety of competitive advantages and applications. In the food industry/functional food/nutraceuticals, the MPMs can be used as a fat replacement agent, as a protein supplement, as a functional food product having a specific

feature (stimulation of the immune system, decreasing levels of triglyceride), as a bio-vehicle for ingredients, flavors, supplements, food additives, vitamins. In the cosmetic and as a cosmeceutical, the MPMs can be used as fat and/or petroleum replacement agent, as a protein supplement in body lotion and cream, as a cosmeceutic product having specific features (increase *in situ* production of collagen), as a bio-vehicle for therapeutic agents, supplements, and vitamins. From the pharmaceuticals point of view, the MPMs can be used as a bio-vehicle for therapeutic agents, to increase oral formulation or generic drugs (excipient), to improve therapeutic indices of drugs (synergy), to reduce drug side effects and to increase bioavailability.

Proteins

Although the preferred source of protein of the invention is the serum lactis, the process can also be applied to diluted protein solution. A variety of proteins are suitable to make the MPM. The term "protein" when used in accordance with this invention means a peptide chain having at least two amino acid residues, preferably at least four, and more preferably more than one hundred amino acid residues. Most preferably the protein is a high molecular weight polypeptide which is preferably water soluble, and may be natural, plant (vegetable) proteins, or animal derived proteins, as well as synthetic proteins.

Examples of natural proteins include albumen, amylase, amyloglucosidase, arginine/lysine polypeptide, casein, catalase, collagen, crystalline, cytochrome C, deoxyribonuclease, elastin, fibronectin, gelatin, gliadin, glucose oxidase, glycoproteins, hexyldecyl ester of hydrolyzed collagen, human placental protein, human placental enzymes, iodized corn protein, keratin, lactoferrin, lactoglobulin, lactoperoxidase, lipase, milk protein, hyristoyl glycine/histidine/lysin polypeptide, nisin, oxido reductase, pancreatin, papaïne, pepsin, placental protein, protease, saccharomyces polypeptides, serum albumin, serum protein, silk, sodium stearoyl lactalbumin, soluble proteoglycan, soybean palmitate, soy, egg, peanut, cottonseed, sunflower, pea, whey, fish, seafood, subtilisin, superoxide dismutase, sutilains, sweet almond protein, urease, wheat germ protein, wheat protein, whey protein, zein, hydrolyzed vegetable protein, and the

like. Preferred is whey which is a mixture of whey proteins obtained from cow's milk following the cheese making.

Synthetic proteins or polypeptides are also suitable. Synthetic proteins are produced by solid phase synthesis, or via recombinant biotechnology processes. MPM can become a solution to the difficulties encountered in the formulation of proteins. MPM can be formulated in creams for instance under either acidic or alkaline conditions without affecting the texture and appearance of the cream.

Polymers

Several polymers may be used in the production of MPMs. They are either synthetic or natural. However a variety of exopolysaccharides and polysaccharides are suitable for the preparation of the MPM used in the compositions of the invention, provided that the exopolysaccharides and polysaccharide contains a sufficient number of hydrophilic groups to cause the resulting MPM. In addition, the polysaccharide must be capable of reacting with the protein to form an MPM having a protein/polysaccharide ratio enough to cause aggregation. The term "polysaccharide" when used in accordance with the invention means a polysaccharide which contains at least four saccharide moieties. The term "saccharide moiety" means a polyhydroxy aldehyde or ketone, or acid hydrolysis product thereof, which, preferably, has the general formula $C_x(H_2O)_y$. Examples of saccharide moieties include the D and L forms of glucose, fructose, xylose, arabinose, fucose, galactose, pyruvic acid, succinic acid, acetic acid, galactose, 3,6-anhydrogalactose sulfate, galactose-4-sulfate, galactose-2-sulfate, galactose-2, 6-disulfate, mannose, glucuronic acid, mannuronic acid, guluronic acid, galactouronic acid, rhamnose, and so on. Preferably the polysaccharides used to make the MPM have molecular weights ranging from about 500 to 15,000,000 daltons, preferably 5,000 to 6,000,000, more preferably 25,000 to 1,000,000 daltons.

These polysaccharides are either added exogenously or produced by a microorganism. Examples of suitable anionic polysaccharides include galactans, galactomannans, glucomannans, polyuronic acids, and the like, which exhibit the requisite number of

pendant hydrophilic groups. Suitable galactans are agar, agarose, kappa carageenan, iota carageenan, lambda carageenan, and the like. Examples of suitable galactomannans are locust bean gum and guar; examples of glucans are cellulose and derivatives thereof, starch and derivatives, dextrans, pullulan, beta 1,3-glucans, chitin, xanthan, tamarind and the like; examples of glucomannans are konjac; examples of polyuronic acids are algin, alginates, pectins; examples of heteropolysaccharides are gellan, welan, gum arabic, karaya gum, okra gum, aloe gum, gum tragacanth, gum ghatti quinceseed gum, psyllium, starch arabinogalactan and so on. Also suitable are dextran sulfate, heparin, pectin, sodium alginate, and mixtures thereof.

These polysaccharides may be further modified as taught in Aoki, T. T.; Araki & M. Kitamikado; 1990, Vibrio sp. AP-2. Eur. J. Biochem, 187, 461-465, provided it contains the requisite number of hydrophilic pendant groups. Also suitable for use in the compositions of the invention are chemically modified galactans, such as those taught in an article authored by K. B. Guiseley in Industrial Polysaccharides;Genetic Engineering, Structure/Property Relations and Applications, Edited by M. Yalpani, 1987, Elsevier Science Publishers. The Guiseley article teaches methods for the chemical modification of agar to obtain optimum gelling properties. In general, any modification of the galactans which does not affect the helical conformation (i.e. which is obtained via linkage of the O6 and O4 of galactose to the O2 of 3,6-anhydrogalactose) will preserve the gelling capability and is suitable for use in the compositions of the invention provided the requisite number of hydrophilic groups are present. The hydrophilic groups provide a polysaccharide which is water soluble. Many other polymers can be added before, during or after the fermentation process. They can be used to change 1) the functional properties of the MPMs, 2) the physical chemistry properties of the MPMs, 3) the aggregation of proteins, 4) the capacity to formulate or encapsulate various components from the various sectors like food, cosmetics, nutraceuticals and pharmaceuticals and 5) the biological activity of the MPMs. Examples of polymers like polyethylene glycol, polyehtyleneimine, polyesters, mono-, di-, or tri-block copolymers or any polymers helping the formation of colloid systems could be used to improve MPMs.

Microorganisms

Although that the preferred microorganism used in the invention is R2C2 (Accession Number 041202-3, National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E 3R2), the process is not limited to one or several specific species or strain and can integrate a variety of other microorganisms either alone or in combination like *Bifidobacterium adolescentis*, *Bifidobacterium angulatum*, *Bifidobacterium animalis*, *Bifidobacterium asteroides*, *Bifidobacterium bifidum*, *Bifidobacterium boum*, *Bifidobacterium breve*, *Bifidobacterium catenulatum*, *Bifidobacterium choerinum*, *Bifidobacterium coryneforme*, *Bifidobacterium cuniculi*, *Bifidobacterium dentium*, *Bifidobacterium gallicum*, *Bifidobacterium gallinarum*, *Bifidobacterium indicum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium longum* DJO10A, *Bifidobacterium longum* NCC2705, *Bifidobacterium magnum*, *Bifidobacterium merycicum*, *Bifidobacterium minimum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium pseudolongum*, *Bifidobacterium pseudolongum subsp. globosum*, *Bifidobacterium pullorum*, *Bifidobacterium ruminantium*, *Bifidobacterium saeculare*, *Bifidobacterium scardovii*, *Bifidobacterium subtile*, *Bifidobacterium suis*, *Bifidobacterium thermacidophilum*, *Bifidobacterium thermacidophilum subsp. suis*, *Bifidobacterium thermophilum*, *Bifidobacterium urinalis*, *Lactobacillus acetotolerans*, *Lactobacillus acidipiscis*, *Lactobacillus acidophilus*, *Lactobacillus agilis*, *Lactobacillus algidus*, *Lactobacillus alimentarius*, *Lactobacillus amylolyticus*, *Lactobacillus amylophilus*, *Lactobacillus amylovorus*, *Lactobacillus animalis*, *Lactobacillus arizonensis*, *Lactobacillus aviarius*, *Lactobacillus bifermentans*, *Lactobacillus brevis*, *Lactobacillus buchneri*, *Lactobacillus casei*, *Lactobacillus cellobiosus*, *Lactobacillus coleohominis*, *Lactobacillus collinoides*, *Lactobacillus coryniformis*, *Lactobacillus coryniformis subsp. coryniformis*, *Lactobacillus coryniformis subsp. torquens*, *Lactobacillus crispatus*, *Lactobacillus curvatus*, *Lactobacillus cypricasei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus delbrueckii subsp. delbrueckii*, *Lactobacillus delbrueckii subsp. lactis*, *Lactobacillus durianis*, *Lactobacillus equi*, *Lactobacillus farciminis*, *Lactobacillus ferintoshensis*, *Lactobacillus fermentum*, *Lactobacillus fornicalis*, *Lactobacillus fructivorans*,

Lactobacillus frumenti, *Lactobacillus fuchuensis*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, *Lactobacillus graminis*, *Lactobacillus hamsteri*, *Lactobacillus helveticus*, *Lactobacillus helveticus subsp. jugurti*, *Lactobacillus heterohiochii*, *Lactobacillus hilgardii*, *Lactobacillus homohiochii*, *Lactobacillus intestinalis*, *Lactobacillus japonicus*, *Lactobacillus jensenii*, *Lactobacillus johnsonii*, *Lactobacillus kefir*, *Lactobacillus kefiri*, *Lactobacillus kefiranofaciens*, *Lactobacillus kefirgranum*, *Lactobacillus kimchii*, *Lactobacillus kunkeei*, *Lactobacillus leichmannii*, *Lactobacillus letivazi*, *Lactobacillus lindneri*, *Lactobacillus malefermentans*, *Lactobacillus mali*, *Lactobacillus maltaromicus*, *Lactobacillus manihotivorans*, *Lactobacillus mindensis*, *Lactobacillus mucosae*, *Lactobacillus murinus*, *Lactobacillus nagelii*, *Lactobacillus oris*, *Lactobacillus panis*, *Lactobacillus pantheris*, *Lactobacillus parabuchneri*, *Lactobacillus paracasei*, *Lactobacillus paracasei subsp. paracasei*, *Lactobacillus paracasei subsp. tolerans*, *Lactobacillus parakefiri*, *Lactobacillus paralimentarius*, *Lactobacillus paraplantarum*, *Lactobacillus pentosus*, *Lactobacillus perolens*, *Lactobacillus plantarum*, *Lactobacillus pontis*, *Lactobacillus psittaci*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus ruminis*, *Lactobacillus sakei*, *Lactobacillus sakei* L45, *Lactobacillus salivarius*, *Lactobacillus salivarius subsp. salicinius*, *Lactobacillus salivarius subsp. salivarius*, *Lactobacillus sanfranciscensis*, *Lactobacillus sharpeae*, *Lactobacillus sp.* NGRI 0001, *Lactobacillus suebicus*, *Lactobacillus thermotolerans*, *Lactobacillus vaccinostercus*, *Lactobacillus vaginalis*, *Lactobacillus vermiforme*, *Lactobacillus versmoldensis*, *Lactobacillus zeae*, *Lactococcus garvieae*, *Lactococcus lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. hordniae*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. lactis bv. diacetylactis*, *Lactococcus piscium*, *Lactococcus plantarum*, *Lactococcus raffinolactis*, *Leuconostoc argentinum*, *Leuconostoc carnosum*, *Leuconostoc citreum*, *Leuconostoc fallax*, *Leuconostoc ficulneum*, *Leuconostoc fructosum*, *Leuconostoc gasicomitatum*, *Leuconostoc gelidum*, *Leuconostoc inhae*, *Leuconostoc kimchii*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Leuconostoc mesenteroides subsp. cremoris*, *Leuconostoc mesenteroides subsp. dextranicum*, *Leuconostoc mesenteroides subsp. mesenteroides*,

Leuconostoc mesenteroides subsp. mesenteroides ATCC 8293, *Leuconostoc pseudomesenteroides*, *Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium australiense*, *Propionibacterium avidum*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionibacterium freudenreichii subsp. freudenreichii*, *Propionibacterium freudenreichii subsp. shermanii*, *Propionibacterium granulosum*, *Propionibacterium jensenii*, *Propionibacterium lymphophilum*, *Propionibacterium microaerophilum*, *Propionibacterium propionicum*, *Propionibacterium thoenii*, *Saccharomyces delbrueckii*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Saccharomyces globosus*, *Saccharomyces carlsbergensis*, *Kluyveromyces fragilis*, *Kluyveromyces bulgaricus*, *Kluyveromyces lactis*, *Torula holmii*, *Candida tenuis*, ES1 (Accession Number 041202-2, National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E 3R2), INIX (Accession Number 041202-4, National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E 3R2) and K2 (Accession Number 041202-1, National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E 3R2). The microorganisms are preferably homolactic but can be heterolactic.

The invention can use various genders and species and examples will be given to demonstrate that they modify functional properties of the MPMs like their hydration and emulsification capacities, their beneficial effects of health or their respective conservation times. MPMs generated with probiotic strain like *Lactobacillus plantarum* allows a better stimulation of the intestinal flora. MPMs generated with *Lactococcus lactis* allow the production of bacteriocin, NISIN, leading to an improved conservation time of the matrices. MPMs generated with *lactobacillus kefiranofaciens* or *L. ramnosus* 9595 allow a better positive overall stimulation of the immune system by their EPS, Murofushi et al. 1986. Immunopharmacology. Vol. 12. pp 29-35. MPM can prolong conservation of products in which it was added or incorporate. Yogurt containing MPM exhibited a better shelf life than the MPM-free yogurt. In addition, MPM can help maintaining survival of microorganisms for a

prolonged period of time. Furthermore, MPM in yogurt serve as a stabilizing agent and replace either gelatine, pectine or corn starch.

In the process of the present invention, the culture of one microorganism can favorise the growth of a second or more microorganism in a sequential fermentation. A first fermentation of the lactic bacteria allows the growth of more demanding bacteria like bifidobacteria and propionibacteria.

The isolation of the bacterial strains (R2C2, K2, ES1) was performed on RCW agars as described by Kojima, S. et al., 1993, Biosci. Biotech. Biochem. Vol. 57, No. 1, pp: 119-120. Kefir grains were homogenized with a blender in an isotonic and sterile solution (tryptone 8,5 g/l + NaCl 1 g/l). This solution was used for RCW agar inoculation.

Different types of colonies were isolated. The selected strains are gram positives, non-mobile, catalase negatives and homofermetative strains. The strains are optionally anaerobic, not growing at 15°C and are having a samel physiology than the species described in Fujisawa et al., International journal of Systematic Bacteriology. Vol. 38. No. 1. pp:12-14. The strains were compared to the reference strain ATCC # 43761 for sugar fermentation pattern as illustrated at Table 1. Moreover, the strains were compared to the reference strains ATCC 43761 and 51647 for 16S homology as shown in Figs. 5A-B. The strains were compared to the reference stain ATCC 43761 for sugar fermentation pattern (as illustrated at Table 1) and to the reference strain ATCC 43761 and 51647 for 16S RNA homology (as illustrated at Table 2). The isolated strains were classified in the genus *Lactobacillus*, and the species *kefiranofaciens*.

Table 1

Fermentation profile of sugar from APA 50 CH and medium API 50 CHL

Substrates	R2C2	INIX	K2	ES1
Glycerol	-	-	-	-
Erythritol	-	-	-	-
D-Arabinose	-	-	-	-
L-Arabinose	-	-	-	-
Ribose	-	-	-	-

Substrates	R2C2	INIX	K2	ES1
D-Xylose	-	-	-	-
L-Xylose	-	-	-	-
Adonitol	-	-	-	-
β-Methyl glycoside	-	-	-	-
Galactose	+++	+++	+++	+++
D-Fructose	+++	+++	+++	+++
D-Mannose	++	+++	+++	+++
L-Sorbose	-	-	-	-
Rhamnose	-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	-
Mannitol	+++	+++	-	+++
Sorbitol	-	-	-	-
α-Methyl-D-Mannoside	-	-	-	-
α-Methyl-D-Glucoside	-	-	-	-
N-acetyl glucosamine	++	++	++	++
Amygdaline	-	-	-	-
Arbutine	-	-	-	-
Esculine	++	-	+++	+++
Salicine	+	++	+++	-
Cellobiose	-	-	+++	-
Maltose	++	+++	-	+++
Lactose	+++	+++	+++	+++
Melbiose	-	-	-	-
Saccharose	+++	++	-	+++
Trehalose	+++	+++	-	+++
Inuline	-	-	-	-
Melezitose	-	-	-	-
D-Raffinose	+	++	-	+++
Starch	-	-	-	-

Substrates	R2C2	INIX	K2	ES1
Glycogene	-	-	-	-
Xylitol	-	-	-	-
β-Gentibiose	-	-	+++	-
D-Turanose	-	-	-	-
D-Lyxose	-	-	-	-
D-Tagarose	-	-	-	-
D-Fucose	-	-	-	-
L-Fucose	-	-	-	-
D-Arabitol	-	-	-	-
L-Arabitol	-	-	-	-
Gluconate	-	-	-	-
2-ceto-gluconate	-	-	-	-
5-ceto-gluconate	-	-	-	-

Factors influencing agglomeration

Salt is one factor for the retrieval of the MPM. In a preferred embodiment, $CaCl_2$ is used but could also be replaced by any salt that is known in the art to have an effect on protein agglomeration like sodium pyrophosphate. For the same purpose, parameters like pH, temperature, enzymatic hydrolysis can be varied to promote agglomeration of proteins to form a matrix.

Food science

A need exists for a polysaccharide produced by a food-grade microorganism, having properties similar to or even superior to xanthan gum. Such an Exopolysaccharide (EPS) can either be added to the food product and the resulting product has to be labeled (but then the product is a so-called "friendly labeled" additive), or it can be produced *in situ* without the necessity of any labeling, because the microorganism is food-grade. The use of such microorganisms for the MPM production is preferred so the MPM produced offer either proteins characteristics and EPS properties. The MPMs can be used as a fat replacement agent, as a

protein supplement, as a functional food product having a specific function (stimulation of the immune system, decreasing levels of triglyceride), as a bio-vehicle for ingredients, flavors, supplements, food additives, vitamins, etc.

Cosmetic

The MPM combined in one matrix polysaccharides and proteins can be used in a wide variety of compositions, including foundation make-ups, skin lotions and creams, sunscreens, blushes, mascara, eye shadows, in addition to hair care products such as shampoos, conditioners, and the like. Suggested ranges of MPM are 0,01-95%, preferably 0,05-50%, more preferably 0,1-30% by weight of the total composition. The composition into which the MPM is incorporated can contain at least one surfactant, which may be an anionic, amphoteric, nonionic, cationic, or zwitterionic surfactant.

The MPM are suitable for use in foundation makeup or color cosmetics such as eye shadow, blush, concealer, or eyeliner compositions in the liquid, cream, solid, or stick form. Suitable compositions may be water-in-oil or oil-in-water emulsions, but are preferably oil-in-water emulsions. Such compositions generally comprise: 0,01-95% MPM, 0,5-95% water, 0,5-25% particulate matter, 0,01-20% surfactant, and 0,1-95% oil. In addition, these compositions may further contain ingredients selected from the group of humectants, preservatives, nonvolatile or volatile oils, gellants, and mixtures thereof.

Nutraceuticals

The MPM of the present invention possesses the synergical sum of the physiological effects of Exopolysaccharides, whey proteins, bacteria, fermentation products that are parts of the MPM, and thus may be used in nutraceuticals.

MPMs have also multiple advantages in the field of probiotics. First, MPMs constitute a mean to produce probiotics at a low cost. During the MPMs retrieval, all bacteria in suspension are retrieved in the MPMs, which represents around 5% of the fermented volume and thus a concentration factor of 20. A fermented solution, containing $1x10^9$ bacteria

generates a concentration of $2x10^{10}$ bacteria in the MPMs. Production of probiotics will at the same time lead to the retrieval of components having health benefits like proteins, peptides and fermentation by-products such as exopolysaccharide, vitamins, bacterial proteins, etc. MPMs constitute also a multifunctional vehicle for probiotics. MPMs allow the incorporation of hydrophobic or hydrophilic substances that an be used to protect, synergize and feed the probiotics. For instance, vitamin C, which is hydrophilic, helps maintaining viability of concentrated probiotics. Presence of certain exopolysaccharides (as for example oligogalactosaccharides) has a prebiotic effect (synergy) on the stimulation of the intestinal flora or the presence of vitamin E (hydrophobic), has protecting effect on the viability of the microorganism (antioxidant) and a nutritive effect (vitamin). In addition, because of its composition in proteins, MPMs are potentially capable of protecting the viability of probiotics. Finally, MPM can serve as a vehicle in different forms, humid, lyophilized or in compressed tablets. All forms constitute advantages in the field of probiotics. Humid MPMs are easy to formulate as shown in food formulations, cosmetics thus suggesting the same for the formulation of probiotics. Lyophilized MPMs, offer an important protection potential because of its content in proteins and the possibility of the incorporation of hydrophilic and hydrophobic protecting substances. Lyophilized MPMs are compressible without the need to add any excipients to form tablets which can be used for incorporation of probiotics or drugs.

Pharmaceuticals

Several drugs may be formulated with the MPM and they may be delivered orally and topically.

A plurality of pharmaceutically related products and drugs or bioactive materials can be formulated with the MPM like small molecules of various classes (hydrophilic and hydrophobic), proteins, RNA, oligonucleotides, DNA, viruses, bacterias. Examples or types of bioactive materials that are used in the MPM and methods of the present invention include any pharmaceutical agents, including, but not limited to anti-inflammatory drugs, analgesics, anti-arthritic drugs, antispasmodics, antidepressants, antipsychotics, tranquilizers, antianxiety drugs, narcotic,

antagonists, antiparkinsonism agents, cholinergic agonists, chemotherapeutic drugs, immunosuppressive agents, antiviral agents, antibiotic agents, appetite suppressants, antiemetics, anticholinergics, antihistaminics, antimigraine agents, coronary, cerebral or peripheral vasodilators, hormonal agents, contraceptives, antithrombotic agents, diuretics, antihypertensive agents, cardiovascular drugs, opioids, and the like.

Suitable bioactive materials also include therapeutic and prophylactic agents. These include, but are not limited to any therapeutically effective biological modifier. Such modifiers include, but are not limited to lipids, organics, proteins and peptides (synthetic and natural), peptide mimetics, hormones (peptides, steroid and corticosteroid), D and L amino acid polymers, oligosaccharides, polysaccharides, nucleotides, oligonucleotides and nucleic acids, including DNA and RNA, protein nucleic acid hybrids, small molecules and physiologically active analogs thereof. Further, the modifiers may be derived from natural sources or made by recombinant or synthetic means and include analogs, agonists and homologs. As used herein "protein" refers also to peptides and polypeptides. Such proteins include, but are not limited to enzymes, biopharmaceuticals, growth hormones, growth factors, insulin, monoclonal antibodies, interferons, interleukins and cytokins. Organics include, but are not limited to pharmaceutically active chemicals with amino, imino and guanidino groups. Suitable steroid hormones include, but are not limited to estrogen, progesterone, testosterone and physiologically active analogs thereof. Numerous steroid hormone analogs are known in the art and include, but are not limited to estradiol, SH-135 and tamoxifen. As used herein, "nucleic acids includes DNA, RNA and physiologically active analogs thereof. The nucleotides may encode single genes or may be any vector known in the art of recombinant DNA including, but not limited to, plasmids, retroviruses and adeno-associated viruses.

The MPMs described in the present invention have an advantage over conventional tablets pill in the above-described patients as it is a non-solid, creamy biodegradable vehicle that can be easily swallowed. Certain polysaccharides found in the MPMs, like kefiran

products by *L. Kefiranofaciens*, are known to pass into blood circulation. These polysaccharides, peptides and bacteria found in the different MPM increase the absorption of some medicaments.

The following non-limiting examples further illustrate the invention and must not be contemplated as to limit the scope of the present invention.

EXAMPLE 1

Preparation of MPM

The preparation of a typical MPM is described in the following example.

Whey obtained from cheddar production is sterilized by filtration (0.22 μm). The sterilized whey is contained in a fermentation chamber at the time of inoculation with the R2C2 strain. A pre-culture is prepared to get a concentration of bacteria of 10^8 to 10^9 per ml of preculture medium. The inoculation is done with a volume of preculture medium (10^8 R2C2/ml) corresponding to 1% and 15% but preferably 10% of the final volume of whey. The fermentation process is done at 37°C and at pH controlled at 5. The pH is controlled by the addition of NaOH. Agitation is maintained to a minimum to allow a uniform distribution but without causing an excessive aeration. The fermentation process is carried out at over a period of 16 to 36 hours depending of the characteristics needed. Following the fermentation process, between 0,1% and 1,5%, but preferably 1% of $CaCl_2$ (w/v) is added and the pH adjusted between 6,5 and 8, but preferably 7,5. The resulting MPMs is malleable, looks like a pudding of white creamy color with no noticeable taste or smell. Retrieval of the matrix is performed by centrifugation. Numerous centrifugal forces are appropriate depending on the functional characteristics desired. However, many tests indicated that a centrifugal force of 3500 RCF (Relative Centrifugal Force) is preferred for the production of a matrix with appropriate functional properties and that a centrifugal force between 3500 and 7476 RCF is also suitable.

An alternative process is using ES1, INIX, K2, R2C2, *Lactobacillus helveticus* ATCC 10386, *Lactobacillus kefirgranum* ATCC

51647, *Lactobacillus ramnosus* ATCC 7469, *Lactobacillus zeae* ATCC 15820 or *Lactococcus lactis* ATCC 11454 and controlling culture conditions of pH and temperature at the controlling values shown in Table 2. An alternative process is adjusting initial pH at the controlling value of microorganism used as shown in Table 2.

Table 2

Parameters for different strains

Strains	Temperature (°C)	pH
ES1	37	5
INIX	37	5
K2	37	5
R2C2	37	5
ATCC10386	42	5,5
ATCC 51647	30	5,5
ATCC 7469	42	6
ATCC 15820	42	6
ATCC 11454	24	6

In another alternative process, the lactoserum is pasteurized before fermentation and the fermentated solution is pasteurized again before separation of the MPMs from the co-products. This alternative however is not used when active bacteria are needed in the final product.

In a further alternative process, a double inoculation is performed with R2C2 and *Lactococcus lactis* to get a co-culture. The two species can also be cultivated together for future innoculation.

In another alternative process, the strain used is *Propionibacterium acidipropionici* ATCC 4875, which produce propionic acid. Fermentation temperature is 30°C, pH is 7, and fermentation is performed for a period of 96 hours. Yeast extracts can be supplemented in proportions of 0.5 to 1% (w/v) to stimulate propionic acid production.

When anaerobic bacteria are used, the process may use addition of gas like CO_2 or nitrogen to remove oxygen and/or increase CO_2 partial pressure.

EXAMPLE 2

Composition of spoonable salad dressing

The matrix is incorporated in the proportions as illustrated in Table 3, for producing a spoonable salad dressing. In this manner, a tasty, creamy and firm dressing similar to mayonnaise is obtained.

Thus, the formulation of the dressing is characterized by the fact that the dressing, when refrigerated at 4°C, keep all is properties. The matrix can replace egg yolks as emulsifiers and stabilizers in oil-water emulsions and the matrix can emulsified as much as its own volume of oil.

The salad dressing is prepared by adding sugar to MPM with agitation to prevent clumping, adding vinegar and corn syrup and stir with an Osterizer blender at maximum speed for 30 seconds, adding corn oil rapidly to the blender jar and maintaining mixing for 1 minute and store salad dressing at 4°C for at least 24 hours.

Table 3

Composition of spoonable salad dressing

	%
MPM	37.6
Salad oil (corn)	37.3
Corn syrup	3.7
Sugar	1.5

EXAMPLE 3

Composition of chocolate milk

A chocolate milk is produced by mixing milk and MPM with an ultraturrax homogenizer, adding dry ingredient, adding liquid ingredients until dry ingredients are completely in solution and refrigerating at 4°C for at least 24 hours. The ingredients are listed in Table 4.

Table 4

Composition of chocolate milk

	%
Milk	53.5
MPM	40.8
Sugar	4.7
Chocolate flavor	0.2
Cocoa	0.1
Dairy Enhancer	To suit 100%

EXAMPLE 4

Composition of light butter

A light butter is produced by softening butter at ambient temperature, mixing butter and MPM, homogenize mixture by using ultra-turrax homogenizer until smooth-mixture is reached and refrigerate at 4°C for at least 24 hours. The ingredients are listed in Table 5.

Table 5

Composition of light butter

	%
Butter	50-85
MPM	15-50

EXAMPLE 5

Use of MPM as thickening agent in yogurt

Yogurt is a dairy product obtained through the fermentation of milk by specific bacterial strains converting part of the lactose into lactic acid such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The milk coagulates when a sufficient quantity of lactic acid is produced.

Since the virtues of yogurt are associated among other things with the bacteria's action in the intestine, their presence in a sufficient

number is important. With respect to this, yogurt should by law, in several countries, contain at least 10 million bacteria per gram at the time it is marketed.

The composition standards stipulate that yogurt must contain not less than 9.5% non-fat milk solids and not less than 3.0% protein. It may also contain some ingredients that come from milk (either whole or skim milk powder, or concentrated evaporated milk), fruits, fruit juices or extracts, jams, cereals or any other flavouring, sweeteners, a quantity not exceeding 2.0% of texturizing agents (stabilizers, gelling, thickening or emulsifying agents), citric acid, food colouring and, in the case of yogurt with added fruit, fruit juices or extracts or jams, a preservative not exceeding 50 ppm.

Potential use of the MPM, as thickening agent in yogurt, may be possible to enhance texture and viscosity in replacement of carrageenan, pectin, gelatin and corn starch among others. The MPM can be of interest as a dairy ingredient in a dairy product since it is a low calorie ingredient.

EXAMPLE 6

MPM as a vehicle to preserve microorganisms alive

MPM can maintains bacteria in life for a long period of time. Three samples of MPM R2C2 (produced with three different centrifuge force: 1592, 3500 and 7476 RCF) were conserved at 4°C during 11 months. After that period of time, a small quantity of MPM was inoculated on RCW agar. The gels were incubated without oxygen at 37°C for 5 days. After incubation, the R2C2 strain was isolated from the samples. It was found that MPM can maintains bacteria in life for a prolonged period of time and therefore can be used as probiotic vehicle.

EXAMPLE 7

Production of MPM containing a probiotic strain

Table 6

Production parameters

Parameters	Lactobacillus rhamnosus 7469
* bacterial count	5,1 x 10^8 b/ml
Lactic acid; t0	0,439 g/l
Lactic acid, End of fermentation	1,67 g/l
Lactic acid in Residual Solution	0,215 g/l
MPM	15,82 g/kg
Total glucide, t0	59,25 g/l
Glucide at the end	51,0 g/l
Glucide in MPM	48,0 g/l
Lactose at T0	45,52 g/l
Lactose at the end	39,68 g/l
Lactose in Residual solution	42,40 g/l
Lactose in MPM	20,70 g/kg
Yield of MPM	28,69 g/l
% humidity	84,67%
% organic matter	9,01%
% minerals	4,95%

* from the culture before retrieval of the MPMs. The count in the MPMs is 20 times higher.

EXAMPLE 8

Makeup production

The MPM as prepared from the method described at the Example 1 was used to prepare two formulations of makeup sticks as follows:

Table 7

Makeup formulations

Formula	Components	w/w %
A	Sodium stearate	7.56
	Water	43.77
	Phenoxyethanol	0.50
	Propyl paraben	0.10
	Methyl paraben	0.30
	Butylene glycol	13.04
	Calcium chloride	0.80
	MPM	0.80
	PEG-20 methyl glucose sesquiisostearate	3.49
B	Hydrogenated castor oil	2.00
	Isostearyl alcohol	5.74
	Titanium dioxide	0.74
	Iron oxide yellow	1.05
	Iron oxide red	0.33
	Iron oxide black	0.13
	Talc	2.23
	Dimethicone	11.63
	Titanium dioxide/trimethylolethane	6.30

EXAMPLE 9

Preparation of a skin lotion

A skin lotion was made according to the following formula:

Table 8

Skin lotion formulation

Components	w/w %
MPM	1.60
Trisodium EDTA	0.10
Butylene glycol	5.00
Sorbitan stearate/sucrose cocoate	6.00
Methyl paraben	0.25
Ethyl paraben	0.15
Xanthan gum	0.30
Octyl methoxycinnamate	7.50
Octyl salicylate	5.00
Benzophenone-3	3.00
C12-15 alkyl benzoate	8.00
Cetyl alcohol	1.00
Phenoxyethanol	1.00
Propyl paraben	0.10
Water	QS

EXAMPLE 9

Encapsulation of trypan blue and fluorescein with MPMs

Marker molecules like trypan blue and fluorescein were encapsulated in MPMs at various concentrations. These formulations were used to perform *in vitro* and *in vivo* experiments to study pharmacokinetic parameters like absorption, bioavailability, distribution, metabolism, and excretion of MPM-based oral formulations. Solubility, stability, liberation were analyzed and compared to the free markers. MPMs were found to have high encapsulation capacity and allow an overall improvement of general pharmacokinetic parameters.

To evaluate the efficacy of a fluorescent probe (Fluorescein (Sigma, Canada)) to penetrate the bloodstream in complexation with a potential drug carrier, female Wistar rats aged 8 weeks were used. The average rat weight was 200 g and they were fed *ad libitum*. Fluorescein (5mg/Kg of body weight) was vortexed either in saline 0,9% or in MPM before gavage. 3 rats/group were fed 1ml of mixed solution via feeding needles at T0 (Ttime). Fluorescein was only present in the first gavage. Rats were gavaged twice a day at an interval of 4 hours. 300µl blood samples were collected at T1, T3,5. When possible, urine was collected before bleedings. Blood was centrifuged at 13000 rpm for 5 minutes. Plasma was collected. 20 µl of plasma were diluted in 2 ml of PBS pH 7,2. Readings were recorded in a spectrofluorometer Eclipse (Varian, Australia) at an excitation wavelength of 490nm and an emission of 514nm. Concentrations were determined against a standard curve of fluorescein. The data below were collected showing that in fluorescein is entering blood circulation slightly better than when formulated in saline and excreted better in animal gavaged with MPMs suggesting a better absorption of fluorescein.

Table 9

Fluorescein concentration in blood over time

Time (hours)	1h (ng/ml)	3h30 (ng/ml)
Saline 0,9%	336,699	99,693
MPM-R2C2	415,701	116,622
MPM-Inix	323,532	84,645

Table 10

Fluorescein concentration in urine over time

Time (hours)	1h (ug/ml)	3h30 (ug/ml)
Saline 0,9%	96,3072	125,6508
MPM-R2C2	176,49423	126,027
MPM-Inix	213,58755	125,0865

EXAMPLE 10

Conditions for industrial production of MPMs

The preculture medium is a medium composed of whey permeate (62,5 g/l) containing 10g/l yeast extract (1%). Sterilized water is used to reconstitute powder whey. Powder of whey permeat is added along with the yeast extract. The medium is pasteurised at 90°C for 30 minutes avoiding the caramel-formation phenomenon and allowing a better ferment growth.

The preculture is generated in fermentor in the following conditions: 39°C, initial pH of 5 is controlled at 4,3 during fermentation, minimal agitation (50 RPM) for 18 to 20 hours with a ratio of initial inoculation between 1 to 15%, but preferably 10% (10^8 bacteria/ml). The inoculation is performed from frozen ferments. The same conditions are used to ferment whey in order to produce the MPMs, but 1% $CaCl_2$ is added to cruce whey, just before the pH adjustment at 5 with HCl. This minimizes the risks of contamination and allows to bring the starting pH close the optimal growth of the bacterial strain used. Once the pH adjusted, whey is pasteurized in the fermentor at 70°C for 40 minutes, temperature is brought to 39 °C and whey is inoculated with 0,5 to 5%, but preferably 2,5% of preculture as defined previously. The fermentation of whey lasts 16 hours with the R2C2 strain. The pH is controlled by adding NaOH. Agitation is maintained to a minimal to allow a good distribution but without causing an excessive aeration. Reajustement of the pH at 7,5 and the retrieval of the MPMs is performed with an industrial clarifier Westfalia model NA-7. The yields obtained depend on the desired firmness and vary between 30 to 50 g/l of fermented solution. The MPM retrieval trigger the recuperation practically of all bacteria found in suspension.

EXAMPLE 11

Inoculation with frozen ferment in whey for MPM production

The MPM was prepared in the same conditions as in the Example 10 except that the fermentation was inoculated without preculture but directly in whey with a frozen ferment. The growth of the ferment is slower and requires a longer fermentation time (24 hours). The quality and

the yield of retrieved MPM in those conditions is comparable to the use of ferment by picking in a preculture as described previously.

EXAMPLE 12

Storage of R2C2 preculture for the MPM production

The preculture of R2C2 as described in the Example 10 can be refrigerated and stored for 2 days without any noticeable alteration. A longer period of refrigeration of 72 hours triggers the apparition of latent phase of the ferment during a subsequent whey fermentation.

EXAMPLE 13

Pasteurization of the fermented whey

Fermented whey can be submitted to a thermal treatment of 65°C for 30 minutes in order to ensure the absence of viable contaminants. The pasteurization of the fermented solution has however to be avoided if ones want to preserve the beneficial effects of the probiotics when used in the fermentation process.

EXAMPLE 14

The use of a clarifier to retrieve the MPMs

Following a fermentation as described in the Example 10 of MPMs, an equipment Westfalia, model NA-7, was tested to continuously retrieve by means of nozzle or to retrieve by periodical discharges. The retrieval with nozzle generates a less dense and liquid MPM. This kind of MPM did not answer the needs for food formulations. In order to increase the density, a short residence time in the clarifier is preferable. This was accomplished by using the periodical discharges. The desired density of MPMs can be adjusted to satisfy the needs for food formulators for different products (butter and cream, etc.). The NA-7 is used at maximal speed of rotation with a flow of 330 L/h of fermented solution and discharged every 7 minutes intervals. The time of aperture of the bowl is adjusted to allow a partial appropriate discharge, eg. allowing neither the MPM accumulation inside the NA-7, nor a complete discharge of the bowl. The yields obtained are 40 to 45 g/L for MPMs having a spreading measurement of 8 to 10 cm while MPM obtained at a lower yield of 30 to

35 g/L for a spreading measurement of 3 cm. All agglomerates and microorganisms in suspension are retrieved.

EXAMPLE 15

Retrieval of MPM with a LAPX404

The LAPX404 machine from ALFA Laval is also utilized at a flow rate of 130 L/h, at a maximal rotation speed of 9500 RPM discharging every 7 minutes. The discharged volume with LAPX404 is partial but constant. In those conditions, density of the MPMs retrieved is appropriate for food formulations. The density can be adjusted by varying the discharges intervals and the flow rate. All agglomerates and microorganisms in suspension are retrieved.

EXAMPLE 16

MPM production from concentrated whey

In the same conditions as described in Example 10, concentrated whey (13% solid matter) is utilized as the fermentation start up solution. In those conditions, yields obtained for MPM production increased to 85 g per liter of fermented solution fermented with the R2C2 strain.

EXAMPLE 17

Protein recuperation by fermentation with specific microorganism

This example describes the use of *Lactobacillus kefirgranum* to help retrieving proteins in fermented solutions. *Lactobacillus kefirgranum* is inoculated from a lyophilised biomass in PL-salt medium as described: Tryptone peptone (casein) 1%, MgSO4 0,02%, MnSO4 0,005%, Sodium acetate 0,2%, Tween 80 0,05%, Yeast Extract 1%, Powder of permeat of whey 62,5g/L. Complete with distilled water, adjust pH between 5,0 and 5,5 with HCl and autoclave at 121°C, 30 minutes. Keep at 4°C until use. Following the fermentation from 24 to 60 hours, preferably 40, at 30°C, the resulting culture can be filtrated or centrifuged or other treatment without any addition in order to retrieve the proteins and the bacteria forming agglomerates.

For the same application, *Lactobacillus kefirgranum* can be cultured in Rogosa Cheese Whey (RCW) medium prepared as follows: RCW is prepared from the Rogosa S1 Broth (Difco # 0478-17-4) and prepared according to the manufacturer's protocols except that distilled water is replaced by whey permeate which is prepared from a powder of whey permeate (62.5 g/L) and the proteins are denatured thermically (Sterilization 121°C for 15 minutes) and fractionated by filtration before use. *Lactobacillus kefiranofaciens* is grown at 30°C for 24 to 60 hours, preferably 40, to allow the retrieval of bacteria and part of the proteins. The culture can be filtered or centrifuged without any other treatment or addition to retrieve the proteins and the bacteria forming agglomerates.

Also, a 400-ml culture in PL-salt, for 24 hours of fermentation at 30°C, is used to inoculate 10 litres of sterile whey. Fermentation is controlled at pH 5,5, 30°C for 24 hours and the fermented solution is centrifuged without any other treatment or addition to retrieve the proteins and the bacteria forming agglomerates. The product retrieved is a MPM at pH 5,5. Another application is to adjust the pH of the fermented solution between 5,5 to 8, preferably at 7,5, before the retrieval of the agglomerates. Finally, another application is to adjust $CaCl_2$ between 0,1 and 1,5 %, preferably at 1%, to the fermented solution before adjusting the pH between 5,5 and 8, preferably at 7,5, and to retrieve the agglomerates.

EXAMPLE 18

MPM as a flavor enhancing agent

In a panel of tasting, the majority of people concluded that the product containing the MPMs had an enhanced flavor. This was reported in butter (the salty taste) and in chocolate prepared drinks (the chocolate taste). Thus, MPM can be used to help increase the flavor of certain product.

EXAMPLE 19

Anti-inflammatory effect of MPMs (Reduction of TNF-α in blood cells)

Female Wistar rats weighing 150 g and fed *ad libitum* were submitted to 3 sessions of gavage (each session lasted a week for 7 repeated gavages) with a week of rest between each session. Blood

samples were collected during and the course of the experiment and the rats sacrificed 2 h after the last gavage for a total of 21 gavages. The samples were harvested (blood cells), on dry ice, and frozen at –80°C. RNA was isolated using TRIZOL reagent (Gibco) as per manufacturers specifications. Ten micrograms of total RNA was reverse transcribed using Superscript RT (Gibco), 500ng oligodT primers (Gibco), and 250ng Random Hexamer primers (Gibco) for 20 minutes at room temperature, followed by 2 hours at 42°C.

TNF-α was amplified from 2ul of reverse transcription reaction using Titanium PCR kit (Clontech). Amplification primers were as follows:

Rat TNF-α forward: CCCAACAA GGAGGAGAGTTCCC (SEQ ID NO:7)

Rat TNF-α reverse: ATGACTCCAAAGTAGACCTGCCC (SEQ ID NO:8)

The PCR reaction was performed for 30 cycles using 100pmole for each primer, with an annealing temperature of 68°C. The PCR products were analysed on a 1.5% agarose gel. The data showed that the MPMs can reduce the level of TNF-α at the RNA level in blood cells after 7 days in the group of rats fed with MPMs in contrast to that observed in the groups fed with saline (- control), HMS90™ (a whey protein isolate sold by Immunotec), yogurt (Danone reduced in calories) therefore suggesting that an anti-inflammatory effect is taking place in blood cells.

EXAMPLE 20

Immunomodulatory effect of MPMs (production of IL-18)

Female Wistar rats weighing 150 g and fed *ad libitum* were submitted to 3 sessions of gavage (each session lasted a week for 7 repeated gavages) with a week of rest between each session. Blood samples were collected during and the course of the experiment and the rats sacrificed 2 h after the last gavage for a total of 21 gavages. The samples were harvested (blood cells), on dry ice, and frozen at –80 degrees. RNA was isolated using TRIZOL™ reagent (Gibco) as per manufacturers specifications. Ten micrograms of total RNA was reverse transcribed using Superscript RT (Gibco), 500ng oligodT primers (Gibco), and 250ng Random Hexamer primers (Gibco) for 20 minutes at room temperature, followed by 2 hours at 42°C.

IL-18 was amplified from 2ul of reverse transcription reaction using Titanium PCR kit (Clontech). Amplification primers were as follows:

IL-18 forward: ATGCCTGATATCGACCGAACA GCC (SEQ ID NO; 9)

IL-18 reverse: CAAATTCCATTTTGTTGTGTCCTG G (SEQ ID NO: 10)

The PCR reaction was performed for 30 cycles using 100pmole for each primer, with an annealing temperature of 68°C. The PCR products were analysed on a 1.5% agarose gel. The data showed that the MPMs increase the levels of IL-18 at the RNA level in blood cells after 24 hours in the group of rats fed with MPMs in contrast to that observed in the groups fed with saline (- control), HMS90 (a whey protein isolate sold by Immunotec), yogurt (Danone reduced in calories) therefore suggesting a stimulation of the mucosal immunity.

EXAMPLE 21

Stimulation of PBMC in rats fed with MPMs

Female Wistar rats weighing 150 g and fed *ad libitum* were submitted to 3 sessions of gavage (each session lasted a week for 7 repeated gavages) with a week of rest between each session. Blood samples were collected during and the course of the experiment and the rats sacrificed 2 h after the last gavage for a total of 21 gavages. Blood samples were measured with Unopett (BD) to count the total peripheral monocular blood cells. The data are shown below and suggest that rats fed with MPMs have a tendency to see their count of PBMC to increase, almost doubled after 4 days post-gavage.

Table 11

PBMC count

Group test	PBMC count increase relative the saline
Saline	1
MPM	1.8
HMS 90	1.2
Yogurt	0.6
Butter	1.2
Butter/MPM (40%MPM)	1.6

EXAMPLE 22

Anti-triglyceridemia effect of MPMs

Female Wistar rats weighing 150 g and fed *ad libitum* were submitted to 3 sessions of gavage (each session lasted a week for 7 repeated gavages) with a week of rest between each session. Blood samples were collected during and the course of the experiment and the rats sacrificed 2 h after the last gavage for a total of 21 gavages. Serum were measured by the method of Wahlefeld (GPO-PAP) to determine the levels of circulating triglycerides. The data of table 12 below shows that MPMs affect the basal level of TG.

Table 12

Triglycerides levels after 24 hours and 3 days

	TG after 24 hours mg/dl serum (SEM)	TG after 3 days mg/dl serum (SEM)
Saline	62 (15)	51 (4)
MPM	33 (5)	32 (10)
HMS 90	31 (4)	101 (15)
Yogurt	118 (38)	76 (27)
Butter	64 (30)	128 (46)
Butter/MPM	83 (9)	156 (22)

Table 13

Triglycerides levels after 24 hours, 16 days and 21 days

Test groups	TG after 24 hours mg/dl serum (SEM)	TG after 16 days mg/dl serum (SEM)	TG after 21 days mg/dl serum (SEM)
Saline	71(14)	80(19)	43(14)
MPM	50(15)	52(1)	35(1)
HMS 90	56(6)	102(34)	92(21)
Yogurt	57(4)	56(13)	99(24)

EXAMPLE 23

Analysis of the amino acid content of MPMs

MPMs were analyzed by Bodycote Canada in order to determine the comparative amino acid content of various whey protein-based product. WPH917 is a high quality whey protein hydrolysate produced by a controlled enzyme treatment of whey protein which provides amino acids, peptides, and polypeptides. Power Pro80 is 80% whey protein concentrate produced by an ultrafiltration process that concentrates native whey proteins.

Table 14

Analysis of the amino acid content

Amino acid	MPM (batch 15)	WPH 917	PowerPro 80
Glutamic Acid	22,92	18,3	10,1
Alanine	5,73	5,2	4,1
Arginine	4,01	3	1,9
Cystine	10,32	2,9	2
Glycine	13,61	2,3	1,5
Histidine	11,17	1,9	1,6
Isoleucine	6,45	5,5	5,1
Leucine	11,89	14,2	9
Serine	7,02	5	3,9
Thryptophane	Not determined	2,3	1,4

EXAMPLE 24

Content analysis of the MPMs

MPMs were analyzed by Bodycote Canada in order to determine the overall content of various whey protein-based products. WPH917 is a high quality whey protein hydrolysate produced by a controlled enzyme treatment of whey protein which provides amino acids, peptides, and polypeptides.

Table 15

Content analysis

Content	Methods	MPM %(g/100g)	WPH917 %(g/100g)
Humidity	AC-HUM 04	80	4
Proteins	AC-PRO01AOAC	8	89
Ashes	AC-CEN01AOAC	6	3.1
Carbohydrates	AC-SUB01AOAC	5	Not determined
Lactose	AC-LAB01AOAC	2.5	0.3
Fat	AC-GRA 01	1.3	3.5
Minerals (Na, Ca, K)	SAA	0.1, 1.8, 0.2	1.3,0.1,002

EXAMPLE 25

Resulting biological effect of pasteutized MPM-INIX on Gluthathione

Female Wistar rats weighing 150 g and fed *ad libitum* were submitted to 3 sessions of gavage (each session lasted a week for 7 repeated gavages) with a week of rest between each session. Blood samples were collected during and the course of the experiment and the rats sacrificed 2 h after the last gavage for a total of 21 gavages. Blood samples were measured after 3 weeks according to a method modified from Anderson designed to measure glutathione levels in plasma.

Table 16

GSH levels in rats

Test groups	Levels of GSH In ug GSH/ml plasma (+/-SD)
Saline	0.05 (0.05)
MPM 1*	0.08 (0.08)
MPM**	1.08 (0.31)

* Plain MPM produced with R2C2 ** Pasteurized MPM produced with INIX

EXAMPLE 26

Solubilization of pyrene using MPM

50 µM pyrene stock solution in acetone is prepared and 20 µL of the stock solution is brought into borosilicate tubes (10 x 13 mm). The tubes are standing at room temperature in a fumehood for 20-30 minutes until complete evaporation of acetone. 2.0 ml of MPM dissolved in 0,1 M phosphate buffered saline (PBS) at pH 7,2 are inserted into the borosilicate tubes. The concentration of MPM used is inside the range of 0,001 to 1,0% (w/v). Let the solution stand at room temperature, protected from the light and fluorescence readings are performed after 24, 48 and 69 h of incubation. Fluorescence reading are performed on a Varian Cary Eclipse at 37°C with an excitation wavelength set at 340 nm and emission scan from 350 to 600 nm are recorded. The degree of solubilization of pyrene is measured by plotting the I_3/I_3(aqueous) of the compound studied as a function of the concentration of the compound. The inflexion point of the plot correspond to the critical micellar concentration value. The data of Table 17 show the ratio of fluorescence intensity of peak I_3 over intensity of peak I_3 from PBS solution as a function of the concentration of MPM and P85.

Table 17

ratio of fluorescence intensity of peak I3 over intensity of peak I3 from PBS solution

	P85	MPM lot 78
0.001%	1,107	1,123
0.01%	1,168	1,328
0.1%	2,564	2,416
1%	8,813	3,687

The data of Table 18 show the ratio of fluorescence intensity of peak I_e over intensity of peak I_e from PBS solution as a function of the concentration of MPM and P85. The data show that MPMs promote the formation of excimers (hydrophobic microdomains).

Table 18

ratio of fluorescence intensity of peak Ie over intensity of peak Ie from PBS solution

	P85	MPM lot 78
0.001%	0,9619	1,567
0.01%	1,233	4,834
0.1%	2,443	30,88
1%	4,829	25,24

EXAMPLE 27

Incorporation of MPM in body lotion

In order to validate the functional properties of the MPMs in a cosmetic formulation, a test was done to formulate a commercial body lotion. The commercial product was a lotion made out goat milk containing 0.3% goat milk extract. The MPM was incorporated in a 10% (w/w). The vessel was closed before mixing the component and agitated by hand and stored at room temperature. After 24 hours, the resulting mixture exhibited fines particles on the vessel walls and was more liquid than the original commercial body lotion. However, the resulting mixture did not show signs of degradation or separation of emulsion and the smell remained similar to the commercial product. Viscosity of MPMs was evaluated using spreading techniques according to the principle of consistometry USDA (Adams & Birdsall, 1946) allowing the measurement, in cm, of the distance of spreading of a semi-fluid food performed on a plate equipped with a series of concentrated circles in predetermined and standardized time. The resulting mixture of body lotion + MPM recorded 13 cm on the spreading scale but get back to its original spreading e.g. 8cm over a period of 3 weeks. The data indicated that the addition of 10% de MPM triggered a liquefaction of the matrice. In terms of shelf-life preservation the resulting mixture was found stable and not contaminated for a period of 3 weeks with the same particle in suspension with the same characteristic smell than the original product.

EXAMPLE 28

Light butter

Two types of light butter were prepared containing 25 and 40% MPM, respectively. In general, the MPM-butter is creamier at room temperature, solidifies less at 4°C, and exhibit a texture which is more breakable than regular butter. It is important to note that the salty taste of butter was enhanced by the MPMs allowing a reduction of the usual amount of salt (NaCl) in the butter by 33 to 50%. The butter containing 25 % MPM is useable for frying and turn brown like plain butter while the 40% butter does not allow frying and is used only as a spread. Also, the conservation time of MPM-butter was acceptable for 45 days and the MPM-containg butter could be frozen without any change of organoleptic properties.

EXAMPLE 29

MPM in whipped cream

Light cream formulations were prepared from 40% cream, milk and MPM to obtain a cream with 21% fat that exhibit a nice and thick texture that can be whipped. The resulting whipped cream is firm and can be stored for 15 days in the fridge without alteration. The odor and taste are similar than the plain cream. The recipe is as follows: 200 ml of cream at 40%, 85 ml milk, 95 g of MPM and sugar and /or aroma.

EXAMPLE 30

Mayonnaise and salad dressing

The same type of MPM used previously was used to prepare Mayonnaise and salad dressing according to the following formula:

Table 19

Salad dressing preparation

Ingredients :	Amount (grams)
MPM	19.6
oil	19.4
Corn syrup	1.9
sugar	0.8
aroma	To taste
spice	To taste
vinegar	10.3

EXAMPLE 31

Chocolate drink

The same type of MPM used previously was used to prepare chocolate drinks according to the following formula:

Table 20

Chocolate drink preparation

Ingredient	Amount in grams
MPM	60
milk	100 ml
cacao	0.16
sugar	7
Chocolate aroma	0.25
Flavor enhancer	To be adjusted

This formulation allows the enrichment of milk of 3,6 g of proteins which is practically doubled as compared to plain milk. The MPM gives a better viscosity and a creamier texture which is appreciated in mouth. MPMs were also added to a weight control drink (Slim fast™) and

the resulting product was found to have a reduced taste of the soy proteins found in that drink.

EXAMPLE 32

Yogurt

Four attempts were made with the formulation of yogurt. (1) control with 3,25% milk and (3) attempts with 1% milk all supplemented with 3% skim milk powder. The steps for the preparation of yogurt included heating the milk at 82-85°C, agitatingh for 30 min, followed with a cooling step at 44-45°C. The culture (yogourmet, LYO-SAN Co) containing *L. Bulgaricus*, *S. Thermophilus* and *L. Acidophilus* were added to get 5 g per liter of milk, followed by an incubation at 45°C for 4 hours, without agitation.

Control test made from 3,25% milk.

Test 2: simultaneously heating of the milk and the MPM (100g/l) followed by the addition of the ferment.

Test 3: the milk is heated first followed by the addition of the MPM (100 g/l) and the ferment.

Test 4: addition of the MPM at the end of the production, at the same time than the addition of the fruits like in an industrial production.

Following the incubation, the product obtained by the Test 2 is comparable to the control test while test 3 and 4 did not allowed the coagulation. After 6 weeks of storage, the control test exhibits a significant drainage and was contaminated with yeast while test 2 did not show neither signs of destabilization nor microbiological deterioration since its preparation. Thus, MPM play a role of preservative on the formulation and helps to obtain very good and typical texture for a yogurt while reducing the amount fat in the final formulation without the addition of starch or other thickening agents.

EXAMPLE 33

The use of different strains affect the analytical profile of the resulting MPMs produced in batches of 10 litres

Concentration of various carbohydrates (glucids), lactic acid and the yields of MPM vary according to the strain used. Also, the composition of the fermented solution influence the composition of the matrix either directly and proportionally like in the case of the carbohydrates found in the aqueous and liquid phase of the matrix, or according to a concentrating effect like in the case of lactic acid that precipitates in part during the retrieval of the matrix.

Table 21

MPM analytical profile in function of different strains used to produce them

	INIX	Lactobacillus rhamno 7469	Lactobacillus zeae	Lactobacillus kefirgranum	Lactobacillus helveticus
*bacterial count	2,47 x 10^8 b/ml	5,1 x 10^8 b/ml	1,34 x 10^8 b/ml	Not determined	6,77 x 10^7 b/ml
Lactic acid ; t0	0,211g/L	0,439g/L	0,143 g/L	0,234 g/L	0,157 g/L
Lactic acid, End of fermentation	2,414g/L	1,67 g/L	0,821 g/L	2,22 g/L	3,5 g/L
Lactic acid in Residual Solution	1,122 g/L	0,215 g/L	0,384 g/L	1,11 g/L	1,53 g/L
MPM	9,57 g/kg	15,82 g/kg	9,85 g/kg	2,94 g/kg	5,23 g/kg
Total **glucide**, t0	45,0 g/L	59,25 g/L	41,30 g/L	54,22 g/L	47,06 g/L
Glucide at the end	41,25 g/L	51,0 g/L	55,01 g/L	51,55 g/L	42,67 g/L
Glucide in Residual solution	42,0 g/L	48,0 g/L	53,86 g/L	46,54 g/L	54,24 g/L
Glucide in MPM	36,50 g/kg	29,35g/kg	39,86g/kg	38,593g/kg	41,197g/kg
Lactose at T0	47,95 g/L	45,52 g/L	53,46 g/L	50,99 g/L	52,58 g/L
Lactose at the end	42,95 g/L	39,68 g/L	51,74 g/L	47,28 g/L	42,65 g/L
Lactose in Residual solution	42,57 g/L	42,40 g/L	47,31 g/L	44,92 g/L	41,55 g/L

Table 21

MPM analytical profile in function of different strains used to produce them

	INIX	Lactobacillus rhamno 7469	Lactobacillus zeae	Lactobacillus kefirgranum	Lactobacillus helveticus
Lactose in MPM	24,78g/kg	20,70g/kg	27,70g/kg	37,02g/kg	35,17g/kg
GALACTOSE at T0	0	0	0	0,13 g/L	0
Galac. At the end	0,75 g/L	0	0	0,75 g/L	2,51 g/L
Galac. in Residual solution	0,70 g/L	0	0	0,36 g/L	2,02 g/L
MPM	1,72g/kg	0	0	1,55g/kg	2,22g/kg
GLUCOSE at T0	0	0	0	0	0
Glucose at the end	0	0	0	0,49 g/L	1,49 g/L
Glucose in Residual solution	0	0	0	0	0,88 g/L
MPM	0	0	0	1,48 g/kg	1,74 g/kg
Yield of MPM	41,2 g/L	28,69 g/L	27,5 g/L	40,6 g/L	40,12 g/L
% humidity	85,98%	84,67%	85,23%	85,11%	
% organic matter	9,49%	9,01%	8,66%	9,87%	
% minerals	4,53%	4,95%	6,12%	5,03%	

* from the culture before retrieval of the MPMs. The count in the MPMs is 5 times higher.

EXAMPLE 34

Characteristics of various MPMs produced from different strains

The use of different strains during the fermentation process affect texture, taste, and smell of resulting MPMs as shown in Table 22.

Table 22

Characteristics of MPM produced from different strains

Parameters	Lactococcuslactis	Lactobacillus helveticus	INIX	Lactobacillus rhamnosus	R2C2
pH	5.69	6.99	6.58	5.06	6,26
Color	7039-12	7042-22	7042-12	7039-12	parsnip
taste	Acre	Acre	Acre	nd	Neutral
smell	Yogurt plain	Condensed milk	Algeas	Strong whey	Cooked cauliflower
Appearence	synerisis important	Nice homogenous texture	Nice soft and viscous texture	bubbles agglomerates	Heterogenous agglomerates
%synerisis	0.07	0.08	0.1	0	0,23
Drainage	nul	nul	nul	nul	nul
***Spreading**	1.25	2	2	5.5	0
** **Emulsifying activity**	Mayonnaise	Vinaigrette	Vinaigrette	Vinaigrette	Vinaigrette

* Viscosity of MPMs was evaluated using spreading techniques according to the principle of consistometry USDA (Adams & Birdsall, 1946) allowing the mesureament, in cm, of the distance of spreading of a semi-fluid food performed on a plate equiped with a series of concentrated circles in predetermined and standadized time.

** The emulsifying activity is evaluated by means of model system representing a good capacity for the formation of a vinaigrette and of and of mayonnaise.

EXAMPLE 35

Characterization and composition of the MPMs produced in 10-litres in fermentors by the R2C2 strain.

Table 33

Characteristics of the MPMs vary according to the growth rate of the strain

Parameters	Sample 1	Sample 2	Sample 7	Sample 8	Sample 7 pasteurized
pH	nd	nd	7.17	7.21	7.06
Color	7042-22	7042-22	7039-12	7039-12	7041-22
Taste	bitter	bitter	bitter	bitter	nd
Odor	Cooked cauliflower	Cooked cauliflower	Cooked cauliflower	Cooked cauliflower	Algae
Appearence	Firm, no synerisis	Yogurt-like synerisis	homogenous soft	homogenous soft	homogenous soft Yogurt-like
%synerisis	0	0.01	0.1	0	0
Drainage	nil	nil	nil	nil	nil
Spreading	nd	nd	8.25	plus de 12	6.5
Emulsifying activity	Vinaigrette	Vinaigrette	Vinaigrette	Vinaigrette	Vinaigrette
Bacterial count in MPM	2×10^9 b/g	$1,4 \times 10^9$ b/g	5×10^8 b/g	$6,7 \times 10^8$ b/g	5×10^8 b/g
Lactic Acid	16,39 mg/g	24,38 mg/g	7,02 mg/g	5,61 mg/g	7,02 mg/g
Total sugars	15,70g/kg	18,30 g/kg	45,19g/kg	43,15g/kg	45,19g/kg
Lactose	0	0	49,88g/kg	48,57g/kg	49,88g/kg
Galactose	14,9g/kg	18,1g/kg	0,66 g/kg	0,77 g/kg	0,66 g/kg
Yield	27,32 g/L	33,85 g/L	41,10 g/L	44,10 g/L	41,10 g/L
% humidity	82,26%	84,82%	86,05%	85,86%	86,05%
% organic matter	10,92%	9,37%	9,01%	9,86%	9,01%
% minerals	6,83%	5,82%	5,08%	4,28%	5,08%

Legend
Nd, not determined
7039-12 white asparagus, off-white with greenish tendency.
7041-12 whiter and less green than 7039-12 (canevas)

7042-12 cauliflower, more beige than white
7042-22 White but with orange background

EXAMPLE 36

Production of MPMs containing proteins from plant origin

The preculture medium is a medium composed of de whey permeat (62,5 g/l) containing 10g/l yeast extract (1%). Sterilized water is used to reconstitute powder whey. Powder of whey permeat is added along with the yeast extract. The medium is pasteurised at 90°C for 30 minutes avoiding the caramel-formation phenomenon and allowing a better ferment growth.

The preculture is generated in fermentor in the following conditions: 39°C, initial pH of 5 is controlled at 4,3 during fermentation, minimal agitation (50 RPM) for 18 to 20 hours with a ratio of initial inoculation between 1 to 15%, but preferably 10% (10^8 bacteria/ml). The inoculation is performed from frozen ferments. The same conditions are used to ferment whey (supplemented with commercial soy proteins 1 to 10% w/v) in order to produce the MPMs, but 1% $CaCl_2$ is added to crude whey, just before the pH adjustment at 5 with HCl. This minimizes the risks of contamination and brings the starting pH close the optimal growth of the bacterial strain used. Once the pH adjusted, whey is pasteurized in the fermentor at 70°C for 40 minutes, temperature is brought to 39°C and whey is inoculated with 0.5 to 5%, but preferably 2,5% of preculture as defined previously. The fermentation of whey lasts 16 hours with the R2C2 strain. The pH is controlled by adding NaOH. Agitation is maintained to a minimum to allow a uniform distribution without causing an excessive aeration. Readjustment of the pH at 7,5 and the retrieval of the MPMs is performed with a, industrial clarifier Westfalia model NA-7. The yields obtained depend on the desired firmness and vary between 30 to 50 g/l of fermented solution. The MPM retrieval trigger the recuperation practically of all bacteria found in suspension.

EXAMPLE 37

Formulation of 5-Fluoro Uracile with the MPMs

MPMs can be used to formulate various bioactive materials. In this example 5-Fluoro Uracile was formulated with the typical MPM described in the above examples and given orally by gavage to mice. The efficacy

on this new formulation was compared to free 5FU and tested in animal models in which colon cancer cells were implanted or in which colon cancer was chemically inducted. The finding was that the 5FU/MPM formulation was improving the therapeutic index of 5FU as shown by reduction of tumor growth.

EXAMPLE 38

Basic formulation of a firm yogurt

1. Add 3 to 5% of mild solids to the milk
2. Heat milk to 85°C and maintain for 30 minutes
3. Reach ebullition and add 0.5% of gelatine, pectine or corn starch to the milk
4. Cool milk to 44-46°C and add bacterial strains converting part of the lactose into lactic acid such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*
5. Add 20% of MPM
6. Place in containers and incubate between 40-46°C until 80°D is reach, for 4-6 hours
7. Refrigerate at 4°C for at least 24 hours.

EXAMPLE 39

Composition of vanilla pudding

A vanilla pudding is produced by mixing milk and MPM, adding dry ingredient to the milk-MPM mixture in mixing well, adding slowly oil to the mixture by using ultra-turrax homogenizer, cooking between 60-70°C until the mixture thickens and refrigerating at 4°C for at least 24 hours. The ingredients are listed in Table 34.

Table 34

Composition of vanilla pudding

	%
MPM	50
Milk	36.1
Sugar	14.9
Vegetal oil	3.3
Na_2HPO_4	0.2
Vanilla	0.5

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A malleable protein matrix comprising:

- a precipitate of a protein of interest in solution;

- at least one microorganism capable of fermenting said solution containing said protein; and

-a matrix carrier allowing fermentation of said protein and said microorganism.

2. The matrix of claim 1, wherein said fermentation is promoted by co-culture of at least two microorganisms simultaneously or successively.

3. The matrix of claim 1, further comprising a fermentation by-products of the fermentation of said solution containing said protein by said bacterial strain.

4. The matrix of claim 1, further comprising peptide.

5. The matrix of claim 4, wherein said peptide comprises at least two amino acid residues.

6. The matrix of claim 4, wherein said peptide comprises more than one hundred amino acid residues.

7. The matrix of claim 1, further comprising components obtained during agglomeration of said protein.

8. The matrix of claim 1, further comprising components present in aqueous phase.

9. The matrix of any one of claims 1 to 8, wherein said protein is selected from the group consisting of natural protein, plant protein, animal derived protein and synthetic protein.

10. The matrix of any one of claims 1 to 8, wherein said protein is selected from the group consisting of albumen, amylase, amyloglucosidase, arginine/lysine polypeptide, casein, catalase, collagen, crystalline, cytochrome C, deoxyribonuclease, elastin, fibronectin, gelatin, gliadin, glucose oxidase, glycoproteins, hexyldecyl ester of hydrolyzed collagen, human placental protein, human placental enzymes, iodized corn

protein, keratin, lactalbumine, lactoferrin, lactoglobulin, lactoperoxidase, lipase, milk protein, hyristoyl glycin/histidine/lysin polypeptide, nisin, oxido reductase, pancreatin, papaïne, pepsin, placental protein, protease, saccharomyces polypeptides, serum albumin, serum protein, silk, sodium stearoyl lactalbumin, soluble proteoglycan, soybean palmitate, soy, egg, peanut, cottonseed, sunflower, pea, whey, fish, seafood, subtilisin, superoxide dismutase, sutilains, sweet almond protein, urease, wheat germ protein, wheat protein, whey protein, zein and hydrolyzed vegetable protein.

11. The matrix of any one of claims 1 to 8, wherein said protein is whey protein.

12. The matrix of claim 3, wherein said fermentation by-products is polysaccharide.

13. The matrix of claim 12, wherein said polysaccharide is selected from the group of exopolysaccharide and anionic polysaccharide.

14. The matrix of claim 12, wherein said polysaccharide contains at least four saccharide moieties.

15. The matrix of claim 14, wherein said saccharide moieties are selected from the group consisting of D and L forms of glucose, fructose, xylose, arabinose, fucose, galactose, pyruvic acid, succinic acid, acetic acid, 3,6-anhydrogalactose sulfate, galactose-4-sulfate, galactose-2-sulfate, galactose-2, 6-disulfate, mannose, glucuronic acid, mannuronic acid, guluronic acid, galactouronic acid, and rhamnose.

16. The matrix of claim 12, wherein said polysaccharide have molecular weight ranging from about 500 to about 15,000,000 daltons.

17. The matrix of claim 12, wherein said molecular weight is ranging from about 5,000 to 6,000,000 daltons.

18. The matrix of claim 12, wherein said molecular weight is ranging from about 25,000 to 1,000,000 daltons.

19. The matrix of claim 12, wherein said polysaccharide is selected from the group consisting of heteropolysaccharide, homopolysaccharide and mixture thereof.

20. The matrix of claim 19 wherein said heteropolysaccharide is selected from the group consisting of gellan, welan, gum arabic, karaya gum, okra gum, aloe gum, gum tragacanth, gum ghatti quicessed gum , psyllium, galactans, galactomannans, glucomannans, polyuronic acids, dextran sulfate, heparin, pectin, sodium alginate and starch arabinogalactan.

21. The matrix of claim 20, wherein said galactan is selected from the group consisting of agar, agarose, kappa, carageenan, iota carageenan and lambda carageenan.

22. The matrix of claim 20, wherein said galactomannan is selected from the group consisting of locust bean gum and guar.

23. The matrix of claim 20, wherein said glucan is selected from the group consisting of cellulose and derivatives thereof, starch and derivatives, dextrans, pullulan, beta 1,3-glucans, chitin, xanthan and tamatind.

24. The matrix of claim 20, wherein said glycomannan is konjac.

25. The matrix of claim 20, wherein said polyuronic acid is selected from the group consisting of algin, alginate and pectin.

26. The matrix of claim 19, wherein said homopolysaccharide is cellulose.

27. The matrix of claim 1, wherein said microorganism is selected from the group consisting of *Bifidobacterium adolescentis*, *Bifidobacterium angulatum*, *Bifidobacterium animalis*, *Bifidobacterium asteroides*, *Bifidobacterium bifidum*, *Bifidobacterium boum*, *Bifidobacterium breve*, *Bifidobacterium catenulatum*, *Bifidobacterium choerinum*, *Bifidobacterium coryneforme*, *Bifidobacterium cuniculi*, *Bifidobacterium dentium*, *Bifidobacterium gallicum*, *Bifidobacterium gallinarum*, *Bifidobacterium indicum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium longum* DJO10A, *Bifidobacterium longum* NCC2705, *Bifidobacterium magnum*, *Bifidobacterium merycicum*, *Bifidobacterium minimum*, *Bifidobacterium pseudocatenulatum*, *Bifidobacterium pseudolongum*, *Bifidobacterium pseudolongum subsp. globosum*, *Bifidobacterium pullorum*, *Bifidobacterium ruminantium*, *Bifidobacterium saeculare*,

Bifidobacterium scardovii, Bifidobacterium subtile, Bifidobacterium suis, Bifidobacterium thermacidophilum, Bifidobacterium thermacidophilum subsp. suis, Bifidobacterium thermophilum, Bifidobacterium urinalis, Lactobacillus acetotolerans, Lactobacillus acidipiscis, Lactobacillus acidophilus, Lactobacillus agilis, Lactobacillus algidus, Lactobacillus alimentarius, Lactobacillus amylolyticus, Lactobacillus amylophilus, Lactobacillus amylovorus, Lactobacillus animalis, Lactobacillus arizonensis, Lactobacillus aviarius, Lactobacillus bifermentans, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus casei, Lactobacillus cellobiosus, Lactobacillus coleohominis, Lactobacillus collinoides, Lactobacillus coryniformis, Lactobacillus coryniformis subsp. coryniformis, Lactobacillus coryniformis subsp. torquens, Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus cypricasei, Lactobacillus delbrueckii, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus delbrueckii subsp. delbrueckii, Lactobacillus delbrueckii subsp. lactis, Lactobacillus durianis, Lactobacillus equi, Lactobacillus farciminis, Lactobacillus ferintoshensis, Lactobacillus fermentum, Lactobacillus fornicalis, Lactobacillus fructivorans, Lactobacillus frumenti, Lactobacillus fuchuensis, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus graminis, Lactobacillus hamsteri, Lactobacillus helveticus, Lactobacillus helveticus subsp. jugurti, Lactobacillus heterohiochii, Lactobacillus hilgardii, Lactobacillus homohiochii, Lactobacillus intestinalis, Lactobacillus japonicus, Lactobacillus jensenii, Lactobacillus johnsonii, Lactobacillus kefir, Lactobacillus kefiri, Lactobacillus kefiranofaciens, Lactobacillus kefirgranum, Lactobacillus kimchii, Lactobacillus kunkeei, Lactobacillus leichmannii, Lactobacillus letivazi, Lactobacillus lindneri, Lactobacillus malefermentans, Lactobacillus mali, Lactobacillus maltaromicus, Lactobacillus manihotivorans, Lactobacillus mindensis, Lactobacillus mucosae, Lactobacillus murinus, Lactobacillus nagelii, Lactobacillus oris, Lactobacillus panis, Lactobacillus pantheris, Lactobacillus parabuchneri, Lactobacillus paracasei, Lactobacillus paracasei subsp. paracasei, Lactobacillus paracasei subsp. tolerans, Lactobacillus parakefiri, Lactobacillus paralimentarius, Lactobacillus paraplantarum, Lactobacillus pentosus, Lactobacillus perolens, Lactobacillus plantarum, Lactobacillus pontis, Lactobacillus psittaci, Lactobacillus reuteri, Lactobacillus

rhamnosus, *Lactobacillus ruminis*, *Lactobacillus sakei*, *Lactobacillus sakei* L45, *Lactobacillus salivarius*, *Lactobacillus salivarius subsp. salicinius*, *Lactobacillus salivarius subsp. salivarius*, *Lactobacillus sanfranciscensis*, *Lactobacillus sharpeae*, *Lactobacillus sp.* NGRI 0001, *Lactobacillus suebicus*, *Lactobacillus thermotolerans*, *Lactobacillus vaccinostercus*, *Lactobacillus vaginalis*, *Lactobacillus vermiforme*, *Lactobacillus versmoldensis*, *Lactobacillus zeae*, *Lactococcus garvieae*, *Lactococcus lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. hordniae*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. lactis bv. diacetylactis*, *Lactococcus piscium*, *Lactococcus plantarum*, *Lactococcus raffinolactis*, *Leuconostoc argentinum*, *Leuconostoc carnosum*, *Leuconostoc citreum*, *Leuconostoc fallax*, *Leuconostoc ficulneum*, *Leuconostoc fructosum*, *Leuconostoc gasicomitatum*, *Leuconostoc gelidum*, *Leuconostoc inhae*, *Leuconostoc kimchii*, *Leuconostoc lactis*, *Leuconostoc mesenteroides*, *Leuconostoc mesenteroides subsp. cremoris*, *Leuconostoc mesenteroides subsp. dextranicum*, *Leuconostoc mesenteroides subsp. mesenteroides*, *Leuconostoc mesenteroides subsp. mesenteroides* ATCC 8293, *Leuconostoc pseudomesenteroides*, *Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium australiense*, *Propionibacterium avidum*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionibacterium freudenreichii subsp. freudenreichii*, *Propionibacterium freudenreichii subsp. shermanii*, *Propionibacterium granulosum*, *Propionibacterium jensenii*, *Propionibacterium lymphophilum*, *Propionibacterium microaerophilum*, *Propionibacterium propionicum*, *Propionibacterium thoenii*, *Saccharomyces delbrueckii*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Saccharomyces globosus*, *Saccharomyces carlsbergensis*, *Kluyveromyces fragilis*, *Kluyveromyces bulgaricus*, *Kluyveromyces lactis*, *Torula holmii*, *Candida tenuis*, R2C2, INIX, ES1 and K2.

28. The matrix of claim 1, wherein said microorganism is selected from the group consisting of *L. rhamnosus, L. acidphilus, L. casei, L. lactis, L. plantarum, L. Kefirgranum,* R2C2, INIX, ES1 and K2.

29. The matrix of claim 1, wherein said microorganism is R2C2 under NML accession number 041202-3.

30. The matrix of claim 1, wherein said microorganism is INIX under NML accession number 041202-4.

31. The matrix of claim 1, wherein said microorganism is *L. Kefirgranum.*

32. The matrix of claim 1, wherein said microorganism is *bacillaceae, bifidobacteriaceae, enterobacteriaceae, enterococcaceae, lactobacillaceae; propionibacteriaceae* and yeast.

33. The matrix of claim 32, wherein said bacillaceae is *Bacillus subtilis.*

34. The matrix of claim 32, wherein said bifidobacteriaceae is one selected from the group consisting of *Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis* and *Bifidobacterium lactis.*

35. The matrix of claim 32, wherein said *enterobacteriaceae* is *Escherichia coli* Nissle 1917.

36. The matrix of claim 32, wherein said *enterococcaceae* is *Enterococcus faecium.*

37. The matrix of claim 32, wherein said *lactobacillaceae* is one selected from the group consisting of *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus crispatus, Lactobacillus fermentum, Lactobacillus johnsonii, Lactobacillus paracasei, Lactobacillus plantarum, Lactobaillus reuteri, Lactobacillus rhamnosus* and *Lactobacillus salivarius.*

38. The matrix of claim 32, wherein said yeast is *saccharomyces cerevisiae boulardii.*

39. A microorganism R2C2 isolated from a consortium obtained from Kefir grain under NML accession number 041202-3.

40. A microorganism K2 isolated from a consortium obtained from Kefir grain under NML accession number 041202-1.

41. A microorganism ES1 isolated from a consortium obtained from Kefir grain under NML accession number 041202-2.

42. A microorganism INIX isolated from ATCC 43761 strain.

43. A process for manufacturing the matrix of claim 1, said process comprising the steps of:

a) fermenting a protein solution with bacteries in a medium;

b) precipitating proteins from the protein solution of step a); and

c) isolating precipitated proteins from supernatant.

44. The process of claim 43, wherein said fermenting step is promoted by co-culturing at least two microorganisms simultaneously or successively.

45. The process of claim 43, wherein said process further comprises a step between steps a) and b) for addition of a polysaccharide.

46. The process of claim 43, wherein said process further comprises a step between steps b) and c) for addition of a polysaccharide.

47. The process of claim 43, further comprising a step of pasteurization of said proteins solution before step a).

48. The process of claim 47, wherein said step of pasteurization is followd by a step of sterilization.

49. The process of any one of claims 43 to 48, wherein precipitation of fermented proteins is effected by at least one method selected from the group consisting of salt addition, pH modulation, thermal treatment, proteolytic enzymes addition and floculent addition.

50. The process of claim 49, wherein said flocculent is a bacterial flocculent.

51. The process of claim 50, wherein said bacterial flocculent is *L. Kefirgranum*.

52. The process of any one of claims 43 to 51, wherein separation of precipitated proteins from supernatant is effected by a method selected from the group of centrifugation and filtration.

53. A composition comprising the matrix of any one of claims 1 to 37 in association with a pharmaceutically acceptable carrier.

54. Use of the matrix of any one of claims 1 to 37, wherein said use is for the manufacture of a product selected from the group of food product, medical product, pharmaceutical product, cosmetic product and nutraceutical.

55. Use of the matrix of any one of claims 1 to 37, wherein said use is for the manufacture of a food product.

56. The use as claimed in claim 55, wherein said matrix is used as an emulsion stabilizer or thickening agent.

57. The use as claimed in any one of claims 55 and 56, wherein said food product is selected from the group consisting of mayonnaise, dressing, margarine, spread, butter, whipped cream and low-fat substitute.

58. The use as claimed in claim 54, wherein said matrix is used as a delivery vehicle.

59. Use of the matrix of one of claims 31 to 37 for the preparation of a probiotic.

60. Use of the matrix of any one of claims 1 to 37, wherein said use is for cosmetic product.

61. The use as claimed in claim 60, wherein said cosmetic product is selected from the group consisting of skin lotion, cream, sunscreen, blush, mascara, eyeshadow, shampoo and conditionner.

62. Use of the matrix of any one of claims 1 to 37 for increasing immune response in a subject.

63. A method of increasing immune response in a subject, comprising administering an effective amount of the matrix of any one of claims 1 to 37 to said subject.

64. Use of the matrix of any one of claims 1 to 37 for reducing triglyceride level in a subject.

65. A method for reducing triglyceride level in a subject, comprising administering an effective amount of the matrix of any one of claims 1 to 37 to said subject.

66. Use of the matrix of any one of claim 1 to 37 for reducing TNF-α level in a subject.

67. A method for reducing TNF-α level in a subject, comprising administering an effective amount of the matrix of any one of claims 1 to 37 to said subject.

68. Use of the matrix of any one of claims 1 to 37 for increasing gluthatione level in a subject.

69. A method for increasing gluthatione level in a subject, comprising administering an effective amount of the matrix of any one of claims 1 to 37 to said subject.

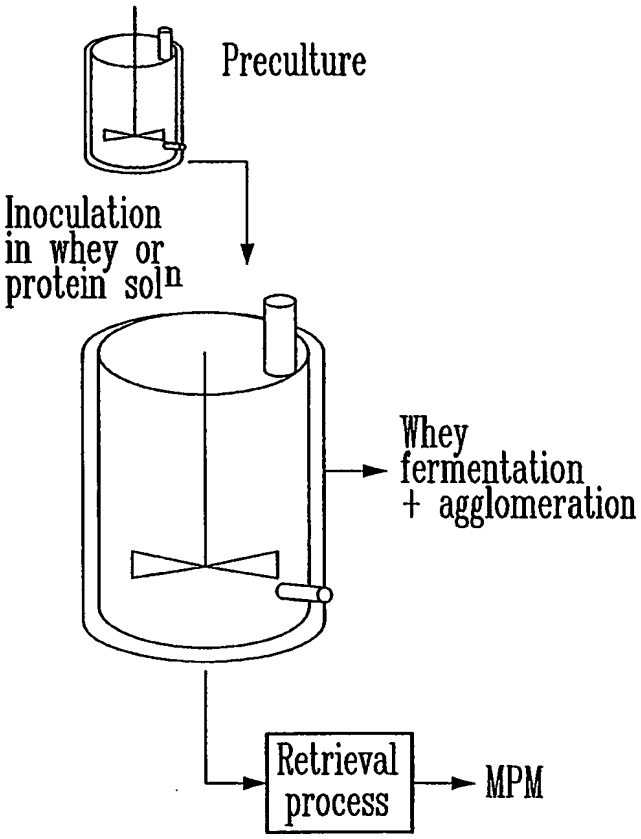
Preculture
Inoculation in whey or protein soln
Whey fermentation + agglomeration
Retrieval process
MPM

Fig. 1

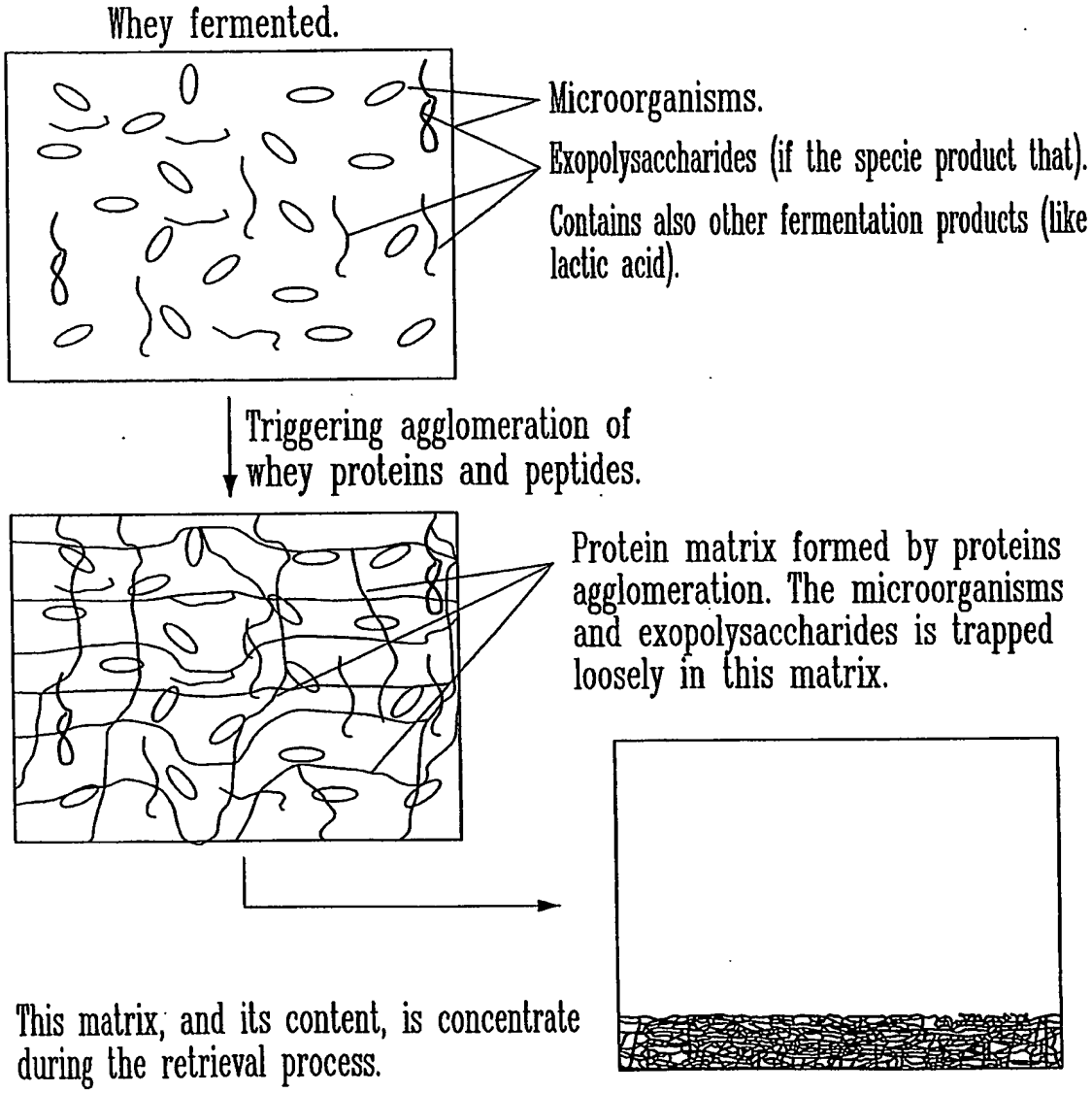
Whey fermented.
Microorganisms.
Exopolysaccharides (if the specie product that).
Contains also other fermentation products (like lactic acid).
Triggering agglomeration of whey proteins and peptides.
Protein matrix formed by proteins agglomeration. The microorganisms and exopolysaccharides is trapped loosely in this matrix.
This matrix, and its content, is concentrate during the retrieval process.

FIG. 2

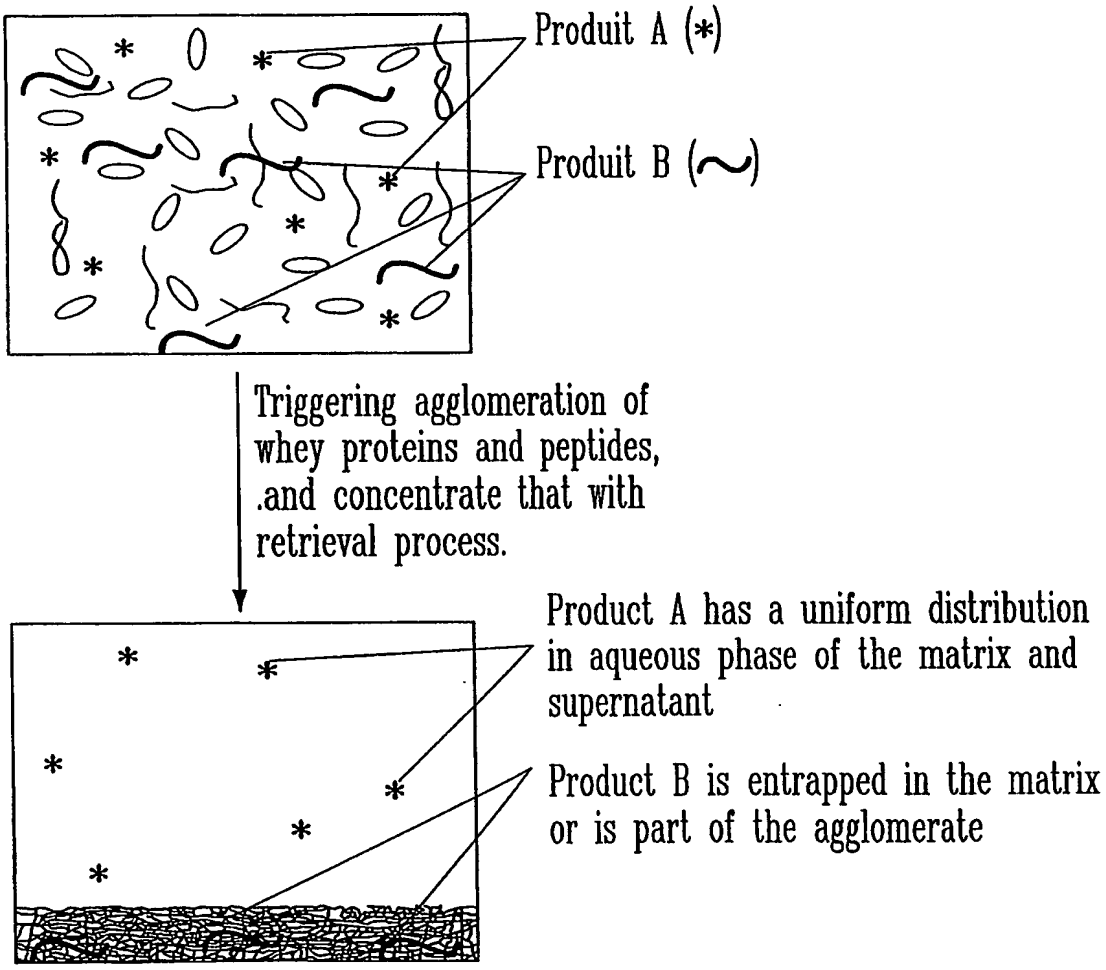
Produit A (*)
Produit B (~)
Triggering agglomeration of whey proteins and peptides, .and concentrate that with retrieval process.
Product A has a uniform distribution in aqueous phase of the matrix and supernatant
Product B is entrapped in the matrix or is part of the agglomerate

FIG. 3

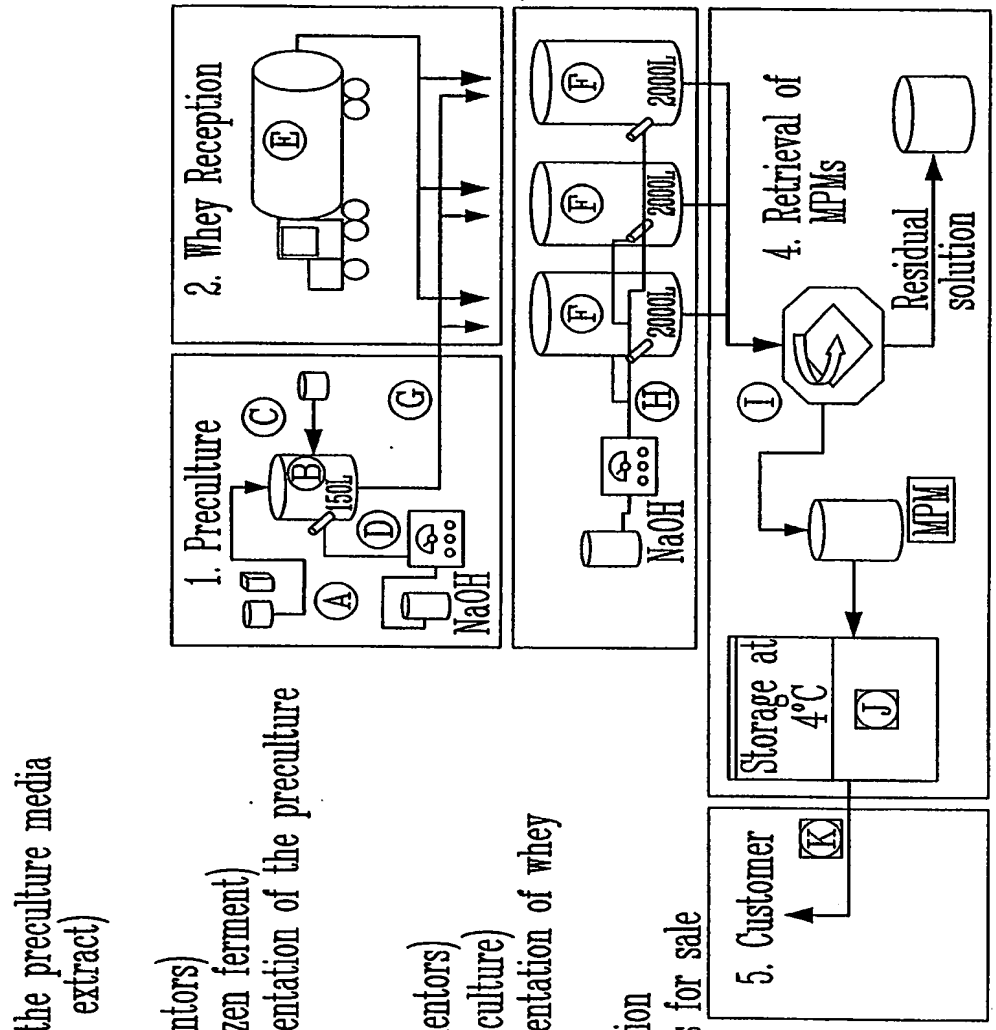
A. Preparation of the preculture media (whey and yeast extract)
B. Pasteurisation (150-liter fermentors)
C. Inoculation (frozen ferment)
D. Controlled fermentation of the preculture
E. Crude whey
F. Pasteurization (2000-liter fermentors)
G. Inoculation (preculture)
H. Controlled fermentation of whey
I. Clarifyer
J. Period of retention
K. Release of MPMs for sale
1. Preculture
2. Whey Reception
NaOH
150L
2000L
4. Retrieval of MPMs
Residual solution
MPM
Storage at 4°C
5. Customer

Fig-4

```
                    1                                                                   75
      INIX    (1) GCAGAATCACTTCGGTGAGGACGCTGGGAAAGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCTTA
        K2    (1) GCAGAATCACTTCGGTGAGGACGCTGGGAAAGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCTTA
      R2C2    (1) GCAGAATCACTTCGGTGAGGACGCTGGGAAAGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCTTA
       ES1    (1) --AGAATCACTTCGGTGAGGACGCTGGGAAAGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCTTA
ATCC 43761    (1) GCAGAATCACTTCGGTGAGGACGCTGGGAAAGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCTTA
ATCC 51647    (1) GCAGAATCACTTCGGTGAGGACGCTGGGAAAGCGAGCGGCGGATGGGTGAGTAACACGTGGGGAACCTGCCCTTA
                   76                                                                  150
      INIX   (76) AGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGTTCGCATGAACAGCTTTTAAAAGGC
        K2   (76) AGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGTTCGCATGAACAGCTTTTAAAAGGC
      R2C2   (76) AGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGTTCGCATGAACAGCTTTTAAAAGGC
       ES1   (74) AGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGTTCGCATGAACAGCTTTTAAAAGGC
ATCC 43761   (76) AGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGTTCGCATGAACAGCTTTTAAAAGGC
ATCC 51647   (76) AGTCTGGGATACCACTTGGAAACAGGTGCTAATACCGGATAAGAAAGCAGTTCGCATGAACAGCTTTTAAAAGGC
                  151                                                                  225
      INIX  (151) GGCGCAAGCTGTCGCTAAAGGATGGACCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCCTACCAAGGCAGTG
        K2  (151) GGCGCAAGCTGTCGCTAAAGGATGGACCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCCTACCAAGGCAGTG
      R2C2  (151) GGCGCAAGCTGTCGCTAAAGGATGGACCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCCTACCAAGGCAGTG
       ES1  (149) GGCGCAAGCTGTCGCTAAAGGATGGACCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCCTACCAAGGCAGTG
ATCC 43761  (151) GGCGCAAGCTGTCGCTAAAGGATGGACCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCCTACCAAGGCAGTG
ATCC 51647  (151) GGCGCAAGCTGTCGCTAAAGGATGGACCCGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCCTACCAAGGCAGTG
                  226                                                                  300
      INIX  (226) ATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA
        K2  (226) ATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA
      R2C2  (226) ATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA
       ES1  (224) ATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA
ATCC 43761  (226) ATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA
ATCC 51647  (226) ATGCATAGCCGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCA
```

FIG. 5A

```
                301                                                                375
      INIX (301) GTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGACCGTAA
        K2 (301) GTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGACCGTAA
      R2C2 (301) GTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGACCGTAA
       ES1 (299) GTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGACCGTAA
ATCC 43761 (301) GTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGACCGTAA
ATCC 51647 (301) GTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGACCGTAA
                376                                                                450
      INIX (376) AGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGG
        K2 (376) AGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGG
      R2C2 (376) AGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGG
       ES1 (374) AGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGG
ATCC 43761 (376) AGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGG
ATCC 51647 (376) AGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTGGCCTTTATTTGACGGTAATCAACCAGAAAGTCACGG
                451                                                                525
      INIX (451) CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
        K2 (451) CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
      R2C2 (451) CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
       ES1 (449) CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
ATCC 43761 (451) CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
ATCC 51647 (451) CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
                526                                                                600
      INIX (526) CAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTTTCTTGAG
        K2 (526) CAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTTTCTTGAG
      R2C2 (526) CAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTTTCTTGAG
       ES1 (524) CAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTTTCTTGAG
ATCC 43761 (526) CAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTTTCTTGAG
ATCC 51647 (526) CAGGCGGAAGAATAAGTCTGATGTGAAAGCCCTCGGCTTAACCGAGGAATTGCATCGGAAACTGTTTTTCTTGAG
```

FIG - 5B

```
                    601                                                                675
      INIX (601) TGCAGAAGAGGAGAGTAGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAATACCAGTGGCGAAG-
        K2 (601) TGCAGAAGAGGAGAGTAGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAATACCAGTGGCGAAG-
      R2C2 (601) TGCAGAAGAGGAGAGTAGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAATACCAGTGGCGAAG-
       ES1 (599) TGCAGAAGAGGAGAGTAGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAATACCAGTGGCGAAG-
ATCC 43761 (601) TGCAGAAGAGGAGAGTAGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAATACCAGTGGCGAAGG
ATCC 51647 (601) TGCAGAAGAGGAGAGTAGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAATACCAGTGGCGAAG-
                    676                                                                750
      INIX (675) CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCC
        K2 (675) CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCC
      R2C2 (675) CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCC
       ES1 (673) CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCC
ATCC 43761 (676) CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCC
ATCC 51647 (675) CGGCTCTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCC
                    751                                                                825
      INIX (750) ATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGCTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCC
        K2 (750) ATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGCTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCC
      R2C2 (750) ATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGCTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCC
       ES1 (748) ATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGCTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCC
ATCC 43761 (751) ATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGCTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCC
ATCC 51647 (750) ATGCCGTAAACGATGAGTGCTAAGTGTTGGGAGGCTTCCGCCTCTCAGTGCTGCAGCTAACGCATTAAGCACTCC
                    826                                                                900
      INIX (825) GCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
        K2 (825) GCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
      R2C2 (825) GCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
       ES1 (823) GCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
ATCC 43761 (826) GCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
ATCC 51647 (825) GCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
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FIG. 5C

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                  901                                                                975
      INIX (900)  TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATTTGTAGAGATACAAAGTCCCTTC
        K2 (900)  TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATTTGTAGAGATACAAAGTTCCTTC
      R2C2 (900)  TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATTTGTAGAGATACAAAGTCCCTTC
       ES1 (898)  TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATTTGTAGAGATACAAAGTTCCTTC
ATCC 43761 (901)  TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATTTGTAGAGATACAAAGTCCCTTC
ATCC 51647 (900)  TTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCTAGTGCCATTTGTAGAGATACAAAGTCCCTTC
                  976                                                               1050
      INIX (975)  GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
        K2 (975)  GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
      R2C2 (975)  GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
       ES1 (973)  GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
ATCC 43761 (976)  GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
ATCC 51647 (975)  GGGGACGCTAAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
                  1051                                                              1125
     INIX(1050)  CGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGG
       K2(1050)  CGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGG
     R2C2(1050)  CGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGG
      ES1(1048)  CGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGG
ATCC 43761(1051)  CGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGG
ATCC 51647(1050)  CGCAACCCTTGTTATTAGTTGCCAGCATTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGG
                  1126                                                              1200
     INIX(1125)  TGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGCACAACGAGCA
       K2(1125)  TGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGCACAACGAGCA
     R2C2(1125)  TGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGCACAACGAGCA
      ES1(1123)  TGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGCACAACGAGCA
ATCC 43761(1126)  TGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGCACAACGAGCA
ATCC 51647(1125)  TGGGGATGACGTCAAGTCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGCACAACGAGCA
```

FIG. 5D

```
                1201                                                                1275
     INIX(1200) GCGAGCCTGCAAAGGCAAGCAAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGA
       K2(1200) GCGAGCCTGCAAAGGCAAGCAAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGA
    R2C2(1200) GCGAGCCTGCAAAGGCAAGCAAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGA
      ES1(1198) GCGAGCCTGCGAAGGCAAGCAAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGA
ATCC 43761(1201) GCGAGCCTGCAAAGGCAAGCAAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGA
ATCC 51647(1200) GCGAGCCTGCAAAGGCAAGCAAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCAGTCTGCAACTCGACTGCACGA
                1276                                                                1350
     INIX(1275) AGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
       K2(1275) AGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
    R2C2(1275) AGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
      ES1(1273) AGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ATCC 43761(1276) AGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ATCC 51647(1275) AGCTGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
                1351                                                        1412
     INIX(1350) ACACCATGGGAGTCTGCAATGCCCAAAGCCGGTGGCCTAACCGCAAGGAAGGAGCCGTCTAA
       K2(1350) ACACCATGGGAGTCTGCAATGCCCAAAGCCGGTGGCCTAACCGCAAGGAAGGAGCCGTCTAA
    R2C2(1350) ACACCATGGGAGTCTGCAATGCCCAAAGCCGGTGGCCTAACCGCAAGGAAGGAGCCGTCTAA
      ES1(1348) ACACCATGGGAGTCTGCAATGCCCAAAGCCGGTGGCC----------
ATCC 43761(1351) ACACCATGGGAGTCTGCAATGCCCAAAGCCGGTGGCCTAACCGCAAGGAAGGAGCCGTCTAA
ATCC 51647(1350) ACACCATGGGAGTCTGCAATGCCCAAAGCCGGTGGCCTAACCGCAAGGAAGGAGCCGTCTAA
```

FIG - 5E

SEQUENCE LISTING

<110> Simard, Eric
Pilote, Dominique
Dupont, Claude
Lajoie, Nathalie
Paquet, Michel
Lemieux, Pierre
Goyette, Philippe
Technologies Biolactis Inc.

<120> Malleable protein matrice and uses thereof

<130> 15468-1pct

<150> US 60/341,232
<151> 2001-12-20

<160> 10

<170> FastSEQ for Windows Version 4.0

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<212> DNA
<213> Artificial Sequence

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<223> 16S gene INIX (Accession number 041202-4, National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E, 3R2

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<210> 2
<211> 1336
<212> DNA
<213> Artificial Sequence

<220>
<223> 16S gene K2 (Accession number 041202-1, National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E,

3R2

<400> 2

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agcagttcgc atgaacagct tttaaaaggc atgcatagcc gagttgagag actgatcggc 180
cacattggga ctgagacacg gcccaaactc ctacgggagg cagcagtagg gaatcttcca 240
caatggacgc aagtctgatg gagcaacgcc gcgtgagtga agaaggtttt cggaccgtaa 300
agctctgttg ttggtgaaga aggatagagg tagtaactgg cctttatttg acggtaatca 360
accagaaagt cacggctaac tacgtgccag cagccgcggt aatacgtagg tggcaagcgt 420
tgtccggatt tattgggcgt aaagcgagcg caggcggaag aataagtctg atgtgaaagc 480
cctcggctta accgaggaat tgcatcggaa actgtttttc ttgagtgcag aagaggagag 540
tagaactcca tgtgtagcgg tggaatgcgt agatatatgg aagaatacca gtggcgaagc 600
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taccctggta gtccatgccg taaacgatga gtgctaagtg ttgggaggct tccgcctctc 720
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caaaggaatt gacgggggcc cgcacaagcg gtggagcatg tggtttaatt cgaagcaacg 840
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ggaaggagcc gtctaa 1336

<210> 3
<211> 1411
<212> DNA
<213> Artificial Sequence

<220>
<223> 16S gene R2C2 (Accession number 041202-3, National
Microbiology Laboratory, Health Canada, 1015

Arlington Street, Winnipeg, Manitoba, Canada, R3E, 3R2

<400> 3

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agcagttcgc atgaacagct tttaaaaggc ggcgcaagct gtcgctaaag gatggacccg 180
cggtgcatta gctagttggt aaggtaacgg cctaccaagg cagtgatgca tagccgagtt 240
gagagactga tcggccacat tgggactgag acacggccca aactcctacg ggaggcagca 300
gtagggaatc ttccacaatg gacgcaagtc tgatggagca acgccgcgtg agtgaagaag 360
gttttcggac cgtaaagctc tgttgttggt gaagaaggat agaggtagta actggccttt 420
atttgacggt aatcaaccag aaagtcacgg ctaactacgt gccagcagcc gcggtaatac 480
gtaggtggca agcgttgtcc ggatttattg ggcgtaaagc gagcgcaggc ggaagaataa 540
gtctgatgtg aaagccctcg gcttaaccga ggaattgcat cggaaactgt ttttcttgag 600
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gttcccgggc cttgtacaca ccgcccgtca caccatggga gtctgcaatg cccaaagccg 1380
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<210> 4
<211> 1384
<212> DNA
<213> Artificial Sequence

<220>

<223> 16S gene ES1 (Accession number 041202-2, National Microbiology Laboratory, Health Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada, R3E, 3R2

<400> 4

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cagttcgcat gaacagcttt taaaaggcgg cgcaagctgt cgctaaagga tggacccgcg 180
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gagactgatc ggccacattg ggactgagac acggcccaaa ctcctacggg aggcagcagt 300
agggaatctt ccacaatgga cgcaagtctg atggagcaac gccgcgtgag tgaagaaggt 360
tttcggaccg taaagctctg ttgttggtga agaaggatag aggtagtaac tggcctttat 420
ttgacggtaa tcaaccagaa agtcacggct aactacgtgc cagcagccgc ggtaatacgt 480
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<210> 5
<211> 1412
<212> DNA
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<220>
<223> 16S gene ATCC 43761

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agcagttcgc atgaacagct tttaaaaggc ggcgcaagct gtcgctaaag gatggacccg 180
cggtgcatta gctagttggt aaggtaacgg cctaccaagg cagtgatgca tagccgagtt 240
gagagactga tcggccacat tgggactgag acacggccca aactcctacg ggaggcagca 300
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gttttcggac cgtaaagctc tgttgttggt gaagaaggat agaggtagta actggccttt 420
atttgacggt aatcaaccag aaagtcacgg ctaactacgt gccagcagcc gcggtaatac 480
gtaggtggca agcgttgtcc ggatttattg ggcgtaaagc gagcgcaggc ggaagaataa 540
gtctgatgtg aaagccctcg gcttaaccga ggaattgcat cggaaactgt ttttcttgag 600
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ggtggcctaa ccgcaaggaa ggagccgtct aa 1412

<210> 6
<211> 1411
<212> DNA
<213> Artificial Sequence

<220>
<223> 16S gene ATCC 51647

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cggtgcatta gctagttggt aaggtaacgg cctaccaagg cagtgatgca tagccgagtt 240
gagagactga tcggccacat tgggactgag acacggccca aactcctacg ggaggcagca 300
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atttgacggt aatcaaccag aaagtcacgg ctaactacgt gccagcagcc gcggtaatac 480
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gtctgatgtg aaagccctcg gcttaaccga ggaattgcat cggaaactgt ttttcttgag 600
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<210> 7
<211> 22
<212> DNA
<213> Artificial Sequence

<220>

<223> Rat TNF-alpha forward primer

<400> 7
cccaacaagg aggagagttc cc 22

<210> 8
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Rat TNF-alpha reverse primer

<400> 8
atgactccaa agtagacctg ccc 23

<210> 9
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> IL-18 forward primer

<400> 9
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<210> 10
<211> 25
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<213> Artificial Sequence

<220>
<223> IL-18 reverse primer

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